bio-techne® / protein simple

Pala Cell Sorter and Single Cell Dispenser

User Guide

Software Version 3.10.0.4

User Guide for Pala Instrument

PN: PL2-0025

Revision B, Jan 2025

For research use only. Not for use in diagnostic procedures

Patents and Trademarks

Bio-Techne's Pala technology is covered by issued and pending patents in the U.S. and other countries. For more information please see **https://www.bio-techne.com/support/instrument-support/intellectual-property**

Chapter 1:
Welcome

Chapter Overview

- Thank You
- Pala Systems
- Pala- Flow Cytometry Combined with Microfluidics
- Optical Layout for the Pala 488/561
- Optical Layout for the Pala 405/488

Thank You

Congratulations on bringing Pala into your lab! We welcome you as a new user and are excited to be a part of your work. This user guide will provide you with details on system hardware, operating the system, how to use Pala software, maintenance procedures and other useful information.

Pala Systems

The Pala system is a microfluidic-based technology for gentle and efficient single cell sorting. The system provides a user friendly interface for easy operation and hassle-free cell sorting with better cell viability and clonal outgrowth for a wide range of cell types, including primary cells, stem cells, and other sensitive cells. This bench top instrument uses proprietary microfluidics technology that combines flow cytometry and liquid dispensing to sort and dispense single cells directly into 96-well or 384-well plates. It simplifies and empowers a number of single cell applications, including cell line development and engineering, single cell genomics, CRISPR editing, antibody discovery, and rare cell isolation such as circulating tumor cells (CTCs) and circulating fetal cells (CFCs). It is affordable, user-friendly, requires no special training to operate, and has simple software-guided maintenance procedures.

- **Single Cell Sorting:** Fluorescent and or non-fluorescent cell populations can be gated and dispensed into a 96-well plate in approximately 2 min or a 384-well plate in 6 min.
- **Bulk Sorting**: Fluorescent and or non fluorescent selected cell population dispensed into a tube.
- **Gentle Sorting:** Low pressure (<2 psi) preserves cell viability and integrity.
- **Fluorescence:** The 2 different Pala systems are configured with 2 lasers: (405nm and 488nm) or (488nm and 561nm).
- **Light Scatter Detection:** 2 light scattering detectors for forward and total light scatter.

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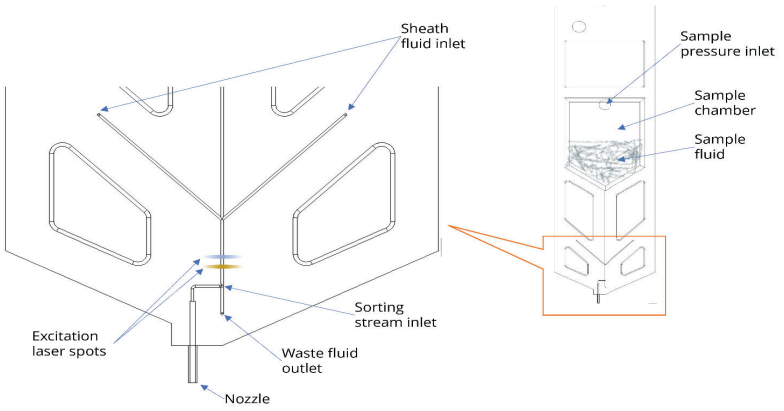
Pala- Flow Cytometry Combined with Microfluidics

Flow Cytometry: The Pala system integrates basic principles of flow cytometry and microfluidics to deliver a platform designed to dispense single cells of specific populations into a microtiter plate or into a single tube. The basic principle behind flow cytometry is the ability to create a uniform stream of cells within a transparent enclosure and exciting the fluorescent stained cells with a laser and detecting the resulting emitted signal. The Pala utilizes a combination of either the 405nm and 488nm or the 488nm and 561nm diode lasers.

Fluorescent dyes are useful since many spectrally distinct dyes can be excited by the use of 2 different lasers. This allows researchers to use up to 9 channels for the 488/561 Pala and up to 11 channels for the 405/488 Pala. The various excitation laser and filter sets allow for a wide variety of fluorescent dyes to be used. The multiple combinations allows for the isolation of a specific cell type based on surface proteins or intracellular organelles/proteins. In addition, the Pala uses the principle of light scattering to differentiate populations of mixed cell types based on size and shape.

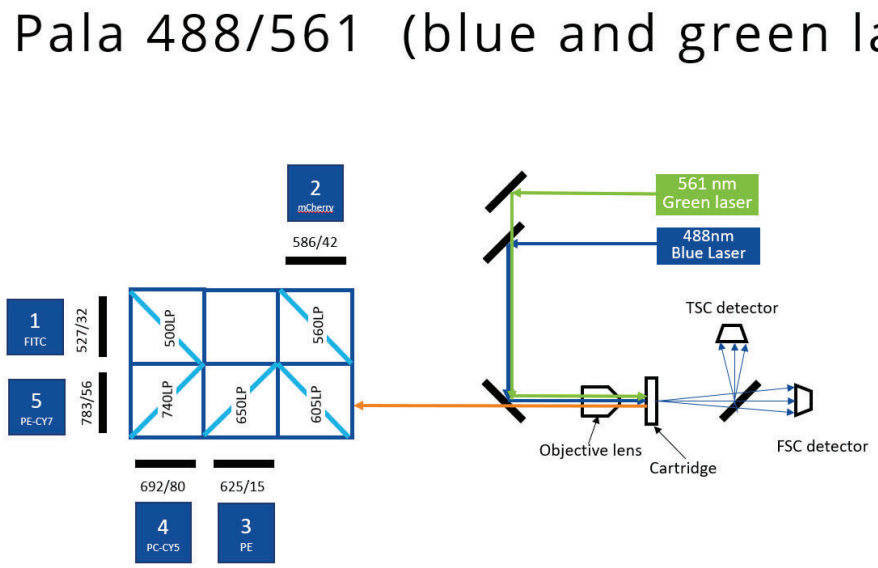
Microfluidics: The Pala utilizes a sterile disposable cartridge for analyzing and dispensing cells. Cells are loaded into the cartridge and the cartridge is placed into a cartridge holder where the sheath and waste fluidic lines are connected to the cartridge through inlet/outlet ports on a manifold. It is also important to note that the sample to be collected for downstream outgrowth or assay is never in contact the instrument itself thus eliminating clogs and maintaining sample to sample independence. The cartridge holder also has a high speed valve that can push a single cell into the nozzle and dispensed into a microtiterplate or tube.

The cartridge is a microfluidic chip designed to focus cells through a channel where the scatter and fluorescence can be interrogated for each cell. The cartridge is connected to two ports where sheath fluid hydrodynamically focuses the cells into a linear stream through the channel. After the cells are interrogated, the gated cell will be directed into a separate channel through the action of the high speed valve. In single cell mode, a gated cell will be ejected through the cartridge nozzle into a well of a microtiter plate with 1 microlter of sheath buffer. In bulk mode, gated cells are directed into the cartridge nozzle with 25-100 nL of sheath buffer per cell. The collection of cells and sheath buffer on the nozzle will form a larger droplet and drip into a microcentrifuge tube on the plate stage. Cells and sheath fluid that are in the channels and are not dispenses through the nozzle are directed to the waste outlet and moved to the waste bottle.

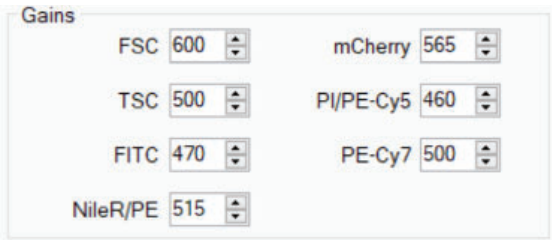


Optical Layout for the Pala 488/561

Below is the top view of the optical layout of the Pala System configured with the 488 and 561 nm diode lasers. Both lasers are directed through a series of dichroic mirrors towards an objective lens which focuses the beam onto the channel where the cells are hydrodynamically focused with sheath fluid. As the cells pass through the channel, the light will scatter and help differentiate cells based on size. The fluorescence emission will be captured with the detectors. Please note that many fluorochromes can be excited by the 488 and 561 nm lasers and a small number of example fluorochromes are listed in this table.

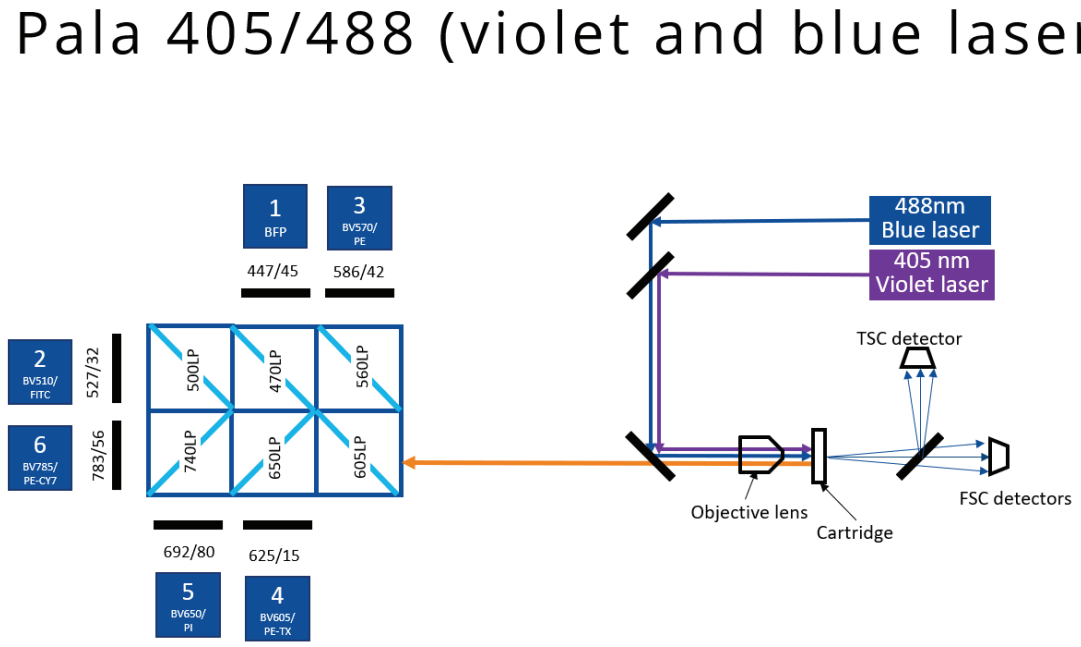


PMT	Filter	488nm Channel Name	561nm Channel Name
PMT 1	527/32	FITC/GFP	
PMT 2	586/42	Nile Red	PE
PMT 3	625/15	488/625	mCherry
PMT 4	692/80	PerCP/PI	PE-CY5
PMT 5	783/56	488/783	PE-CY7

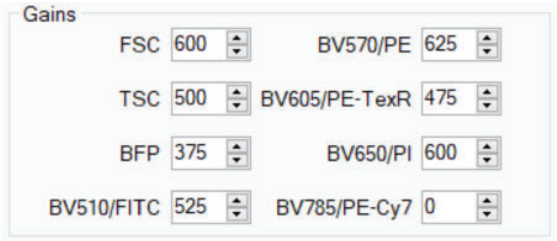


Optical Layout for the Pala 405/488

Below is the top view of the optical layout of the Pala System configured with the 405 and 488 nm diode lasers. Many fluorochromes can be excited by the 405 and 488 nm lasers and only a small representation of fluorochromes are listed in this table.



PMT	Filter	405nm	488nm
PMT 1	447/45	BFP	
PMT 2	527/32	BV510	FITC/GFP
PMT 3	586/42	BV570	PE/DsRed
PMT 4	625/15	BV605	PE-Texas Red
PMT 5	692/80	BV650	PerCP/PI
PMT 6	783/56	BV785	PE-Cy7



Chapter 2:

Getting Your Lab Ready

Chapter Overview

- Introduction
- Whats in the Box
- Additional Useful Items - Not Supplied
- Physical Specifications
- Electrical Requirements
- Software and Workstation Requirements
- Laser Information
- Moving the Pala- Carefully
- Sheath and Waste Bottles
- Cell Cartridges
- Cartridge Holder
- System Sterilization

Introduction

This chapter will help you prepare the lab for Pala 488/561 or your Pala 405/488. Please have the space, electrical and environmental requirements ready prior to scheduling your installation.

NOTE: Please wait for an authorized ProteinSimple Field Application Scientist to unpack and install Pala for you. Don't try doing this yourself. Handling Pala incorrectly could cause damage to the system.

Whats in the Box

Carefully inspect all boxes upon receipt of the single cell dispenser. If there are any signs of mishandling or damage, file a claim with the carrier immediately. If the shipment is separately insured, please file a claim with the insurer.

Components	QTY
Pala Instrument 405/488 or 488/561	1
Sheath Bottle	1
Waste Bottle	1
Cleaning Cartridge	1
Power Adapter for Pala	1
Cleaning Tray	1
USB 2.0 A-B Cable	1
Microcentrifuge Tube Adapter	1
PCR Plate Adapter	1
Sterile Cleaning Cotton Swabs	5
Flashlight	1
Laptop Workstation	1
Mouse	1
Laptop Power Adapter	1
Quick Start Guide	1

Additional Useful Items - Not Supplied

- Single Cell and Bulk Cell Cartridges. These disposable cell cartridges are specially designed by Bio-Techne to achieve optimal dispensing operation, accuracy, and to avoid sample cross contamination.
- Microsoft® Office™ or equivalent.
- Anti-virus software. This system is intended for use as a stand-alone station, not as a part of a network
- PBS, sterile double distilled Water (ddH2O)
- Control beads
- Basic lab supplies, pipettes, tips, 96-well or 384-well tissue culture plates or PCR plates
- Fluorescent Microscope

Space Requirements

You will need a lab bench or table that can support 40 lb (18 kg) and has enough space for both Pala and the laptop workstation. The single cell dispenser is designed to fit on a laboratory bench top or in a tissue culture hood. Take special care while handling fluids around the dispenser. Avoid spills around the system. Never place anything on top of the dispenser. Turn off the instrument and unplug the power cord and USB cord before manually cleaning the instrument with ethanol wipes.

IMPORTANT
Pala needs a stable surface and must remain level to work properly. The unit can be placed on a bench, table or tissue culture hood. If the instrument is placed in a tissue culture hood, the laptop can be placed outside on a small table.

Dimension	Meters	Feet
Width	0.9	3.0
Depth	0.8	2.5
Height	0.9	3.0

Minimum recommended space requirements for Pala.

Physical Specifications

Description	Specification
Pala Dimensions	23 cm x 65 cm x 36 cm (H x W x D) 9″ x 25″ x 14‴ (H x W x D)
Pala Weight	15.8 kg (35 lb)

For indoor use only. Use up to altitudes of 1524 meters (5000 feet).

Environmental Requirements

Requirement	Specification
Operating temperature range	8–25 °C (64–77 °F)
Operating humidity range	20–80% relative, non-condensing

Electrical Requirements

Pala requires a dedicated, grounded circuit capable of delivering the appropriate current and voltage for your country. The power requirements for the 2 Pala systems are 100 V–240 V (AC), 50/60 Hz, 20 W.

Software and Workstation Requirements

The Pala is supplied with a laptop workstation with the Pala software and drivers installed and configured at the factory. Due to the rapid changes and advances in laptop workstation design, the minimum provided specifications are listed below.

Component	Minimum Requirements
Operating System	Windows 11
Processor	Intel Core i7, 13 Gen
Memory	16 GB
Disk Space	512 GB SSD
USB Ports	1 USB 3.0, 1 USB-C
Screen Size	15.6”

Laser Information

The Pala uses either a combination of 405 and 488 nm lasers or 488 and 561 nm lasers. All supplied lasers are Class 3 lasers that are embedded internal to the instrument for cell detection. Any hazardous light emission from the lasers are not accessible from the outside of the product during normal use. Do not open or remove the instrument enclosure as this may result in hazardous radiation exposure. Do not operate the instrument if there is any significant damage to the enclosure.

Class 1 laser emissions will be emitted out of the top of the product and the side, during normal use which are safe to the eye. The Pala complies with FDA performance standards for laser products except for deviations pursuant to Laser Notice No. 50, dated 6/24/2007 and Laser Notice 56, draft dated 1/19/2018 and complies with IEC/EN 60825-1:2014 and 2007.

WARNING: Use of the Pala outside of procedures described herein may result in hazardous radiation exposure. Under no circumstance should the enclosure be removed unless by a certified service engineer.

Moving the Pala- Carefully

Take all standard precautions when moving the Pala System. The Pala has a sensitive internal optical bench with lasers, detectors, and lenses that can become misaligned if not properly packaged during shipment or movement from one bench to another bench. When lifting the Pala, lift from the corners of the instrument and avoid lifting from the bottle holder.

Sheath and Waste Bottles

Sheath and waste bottles must sit in the liquid level detection tray while instrument is in use. The bottles sit on a calibrated scale and a software prompt is triggered if the sheath volume is too low, the waste volume is too high, or if the bottles are removed from the tray during use.

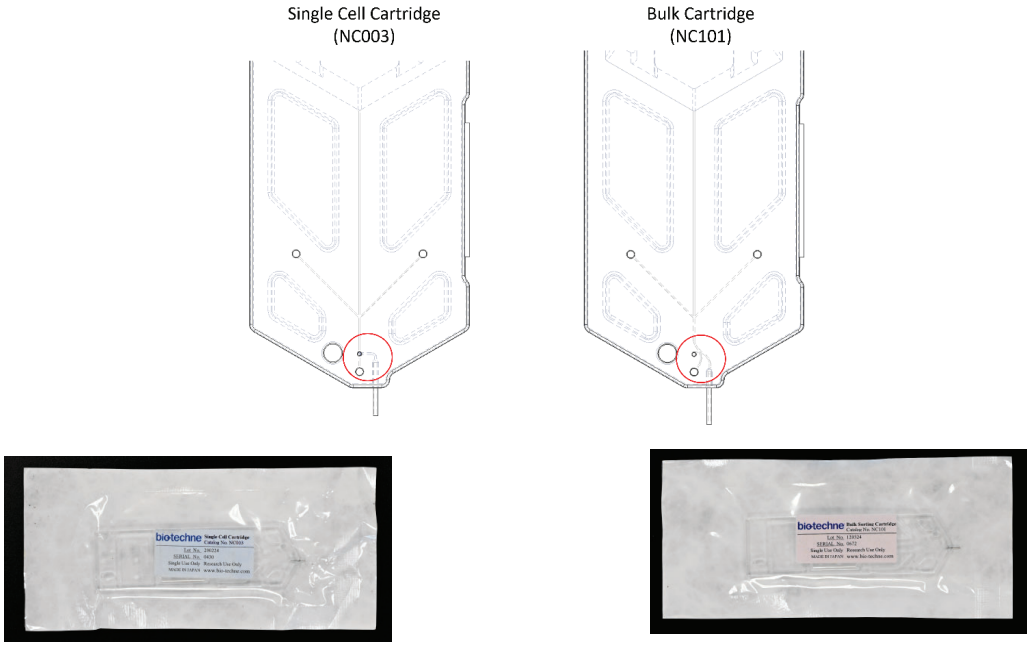
Sterile distilled, filtered water or sterile PBS have been successfully tested as sheath buffer. Media and other buffers have not been optimized. Users may utilize other sheath buffers at their discretion but should optimize these on a case by case basis. Since the sheath buffer is running through microfluidic channels in the cell cartridge, it is recommended that buffers are filtered through a 0.2 uM filter prior to use. Additionally, all buffers should be removed and the instrument flushed with sterile distilled water during instrument shutdown, prior to storage, to prevent salt deposit build up in the fluidic lines.

Additional sheath bottles can be purchased and can be filled with distilled water or PBS and autoclaved if a sterile application is needed.

CAUTION: Sheath and waste bottles are under pressure. All microfluidic lines and bottle lids must be secured for the instrument to function properly. Do not let the waste bottle exceed 200 ml. If waste overflows, it can be siphoned into the vacuum line and the water will damage the fluidic lines in the instrument.

Cell Cartridges

The Pala instruments utilize two types of cell cartridges for performing aseptic single cell dispensing, bulk sorting, and rare cell enrichment. Single cell cartridges and bulk sorting cartridges are uniquely designed for their specific purpose and should not be used interchangeably as this will result in the system not performing according to its specifications and may result in possible instrument clogging or failure. Please note that both cartridge types are very similar in appearance.



Description	Single Cell Cartridge NC003	Bulk Sorting Cartridge NC101
Sample Reservoir	Up to 600 uL	Up to 600 uL
Max Sample Flow Rate	23 uL/min	23 uL/min
Sheath Flow Rate	6.5ml/hour or 108 uL/min	6.5ml/hour or 108 uL/min
Sorting Pressure	<2 PSI	<2PSI
Recommended Cell Density Range	5,000-20,000 cells/mL	100,000-500,000 cells/ml
Recommended Sorting Speed	1-2 events/second	<50 events/second
Volume Per Cell	1 uL	25-100 nL
Purity	N/A	80-90%
Accuracy	>95% single cells/well	N/A
Number of Sorted Cells	Up to 3,072 cells/run	Up to 65,000 cells/run

Cleaning Cartridge

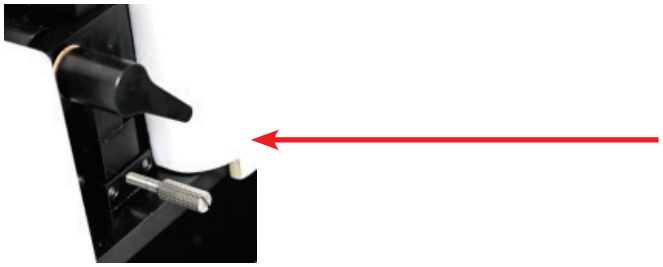
The cleaning cartridge is a specifically designed cartridge to enable instrument initialization, priming, and shutdown. The cleaning cartridge should be kept in the cartridge holder when instrument is not in use and should be kept dry and free of dust, oils, and other contaminants. Cleaning cartridges may be wiped with 70% ethanol and a soft tissue to clean. It is different from the Single Cell and Bulk Cartridge in that it does not have a stainless steel nozzle, sample reservoir, and the fluidics only connect the sheath fluidic lines to the waste line. The cleaning cartridge should be replaced if significant excessive wear is noticed for if the fluidic ports are clogged.

Cartridge Holder

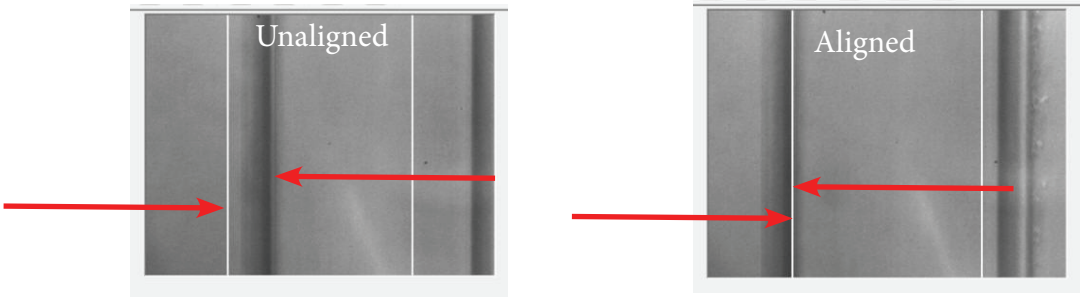
The cartridge holder is designed to hold the cell cartridges in position to enable the instrument to properly run the fluidics, align the cartridge with the instrument optics, and position the dispense nozzle for accurate dispensing. The Pala instrument automatically locks and unlocks the cell cartridges in place during initialization, setup and shutdown. Do not manually turn the lever to open or close the cartridge holder.

Warning: Never manually unlock the cartridge holder locking mechanism while the instrument pump is running. This will result in a system leak that will require additional cleaning and instrument priming.

Cartridges loaded into the cartridge holder may need a slight alignment so that the lasers are properly focused onto the channel that directs cells to the sorting junction. The cartridge holder has a stainless steel positioner knob that moves the cartridge from side to side so that lasers are focused directly onto center of the channel. After insertion of a single cell or bulk cartridge into the cartridge holder during setup, a live camera image of the channel will appear.



Align the image so that the dark line of the channel aligns to the left white line in the image. Once aligned, click OK to proceed.



System Sterilization

The Pala instrument was developed to enable aseptic technique for single cell dispensing and bulk sorting. The Pala is portable and can be used inside a biosafety cabinet. If aseptic technique is required for a specific experiment, we recommend following the System Sterilization protocol. Ensure to plan approximately 1 hour to perform the system sterilization. The instrument can remain in the bio safety cabinet and re-sterilized as needed. This is a closed system so after sterilization, the instrument can be stored outside of the hood without loss of sterility. Additional information regarding the sterilization process is further detailed in **Chapter 08: Appendix- Sterilization**.

Chapter 3:

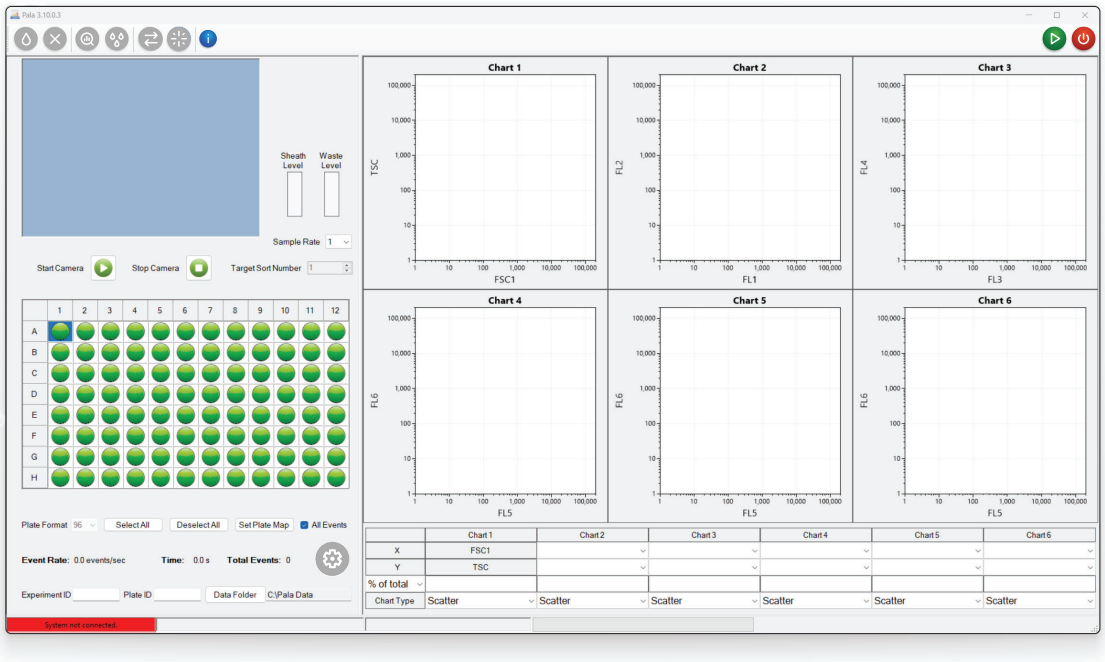
Software Commands and Interface

Chapter Overview

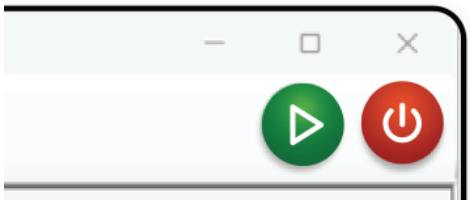
- Overview
- Changing Chart Type
- Accessing the Chart Menu & Creating Polygon Gates
    - Adding Vertices to Polygon Gates (3-8 Vertices are supported for a gate)
    - Saving Gates
- Quadrant Gates
- Histogram Gating
- Histogram and Density Plot Bin Size
- Log/Linear Scaling and Chart Interaction.
- Chart Navigation Commands
- System Control Menu
    - Plate Options
    - Sample Rate
    - Pala Data and Log Files

Overview

This section of will cover the software layout, keyboard shortcuts and functions. The Pala instrument must be turned on before launching the software by depressing the power button on the front of the instrument. A series of 2 chimes will originate from the laptop confirming that the instrument is properly communicating and connected with the laptop. At this point, the Pala executable located on the desktop can be launched.



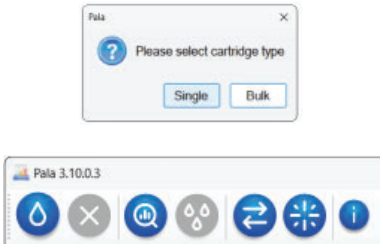
Pala Control Icons



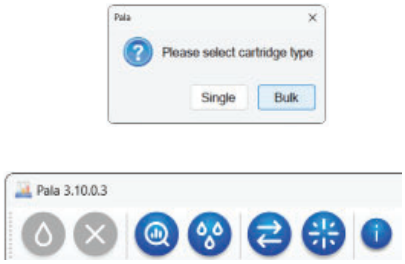
Initialization Icon: Initiates the priming of the Pala instrument. This operation will take about 5 minutes to complete and will use about 20 mLs of sheath fluid for this process.

Shutdown Icon: After completion of experiments, clicking the shutdown icon will initiate a series of tasks and prompts including a fluidic line flush for the user to successfully shutdown the instrument.

IMPORTANT: If anything other than water was used as the sheath fluid, sterile distilled water must be used at the end of the shutdown process. PBS or any other saline based sheath fluid can crystallize and potentially clog the fluidic lines in the instrument if water is not used during the shutdown process.



Task bar options when **Single Cartridge** type is selected



Task bar options when **Bulk Cartridge** type is selected

Single Dispense Icon: This icon will initiate the dispensing of single cells into a 96 or 384 well plate. For dispensing, at least one gate must be drawn on a chart and wells must be selected on the plate map. This icon will turn green during single cell dispensing.

Cancel Action Icon: When the instrument is dispensing in single cell mode, the action can be canceled at any time by clicking on this icon immediately right of the Single Dispense Icon.

Analysis Icon: Clicking this icon will turn on the lasers and detection channels for determining the optimal gating parameters for selecting cells to dispense. This icon will turn green during analysis.

Bulk Sorting Icon: Once analysis is performed, gates are drawn, and a target cell number is added, this icon will initiate the sorting of cells into a microcentrifuge tube. This icon will turn green during bulk sorting.

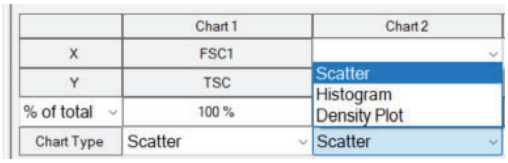
Switch Icon: Clicking this icon initiate instructions to switch cartridges in the cartridge holder. The action prompts the user to insert the Cleaning cartridge to re-prime the system prior to inserting a new cell cartridge.

Cleaning Icon: Clicking this icon initiates a procedure to prepare the Pala for aseptic sorting. Reagents such as 10% bleach, 70% ethanol, sterile filtered ddH20, and sterile swabs are necessary for completing this procedure.

Information Icon: Clicking this icon will display the loaded software and firmware version.

Changing Chart Type

Select the desired chart type (Scatter, Histogram, Density) in the bottom tool bar by clicking on the "Chart Type" drop down below each specific chart. The X and Y axis can be configured using the fluorescent or scatter detectors as well.



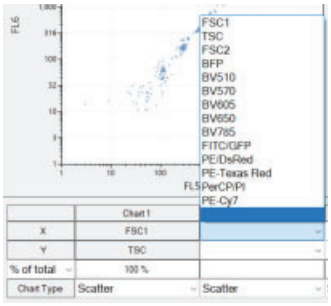
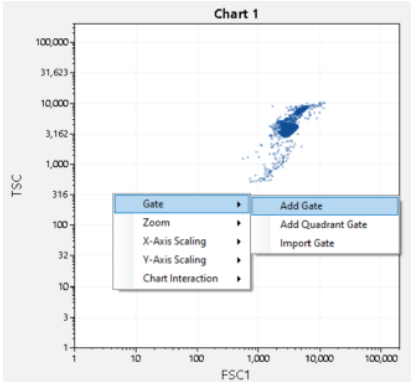
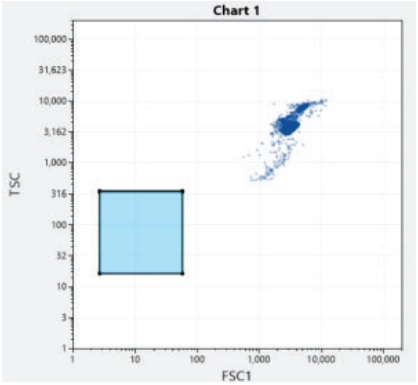


	Chart 1	Chart 2	Chart 3	Chart 4
X	FSC1	FITC/GFP	FITC/GFP	FITC/GFP
Y	TSC	PE/DsRed	FSC1	
% of total	89.79 %	29.73 %		
Chart Type	Scatter	Scatter	Density Plot	Histogram

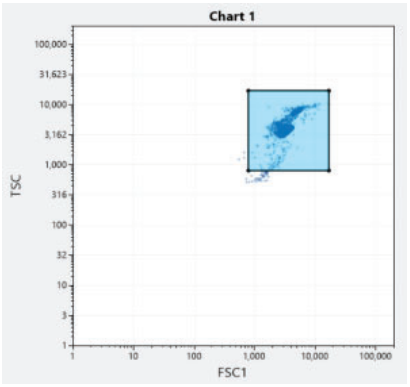
Accessing the Chart Menu & Creating Polygon Gates

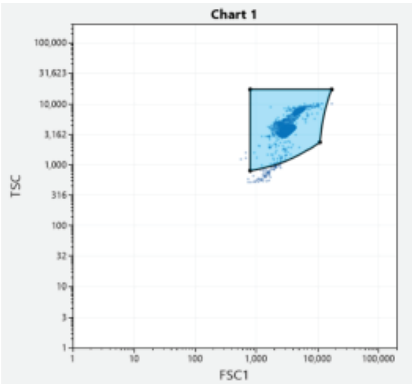
- Right click on any area of the chart to display the chart menu
- Hover over "Gate" and left click "Add Gate"
- To move the gate to a desired location, hold Ctrl + Left click on gate and drag
- Hover over any vertex and Left click when "pointer" appears, hold and drag to adjust the gate's size and shape





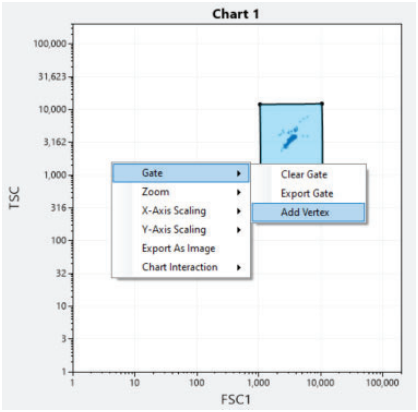
- To rotate and resize gate, hover over any vertex, when finger appears, hold Shift + Left click and drag

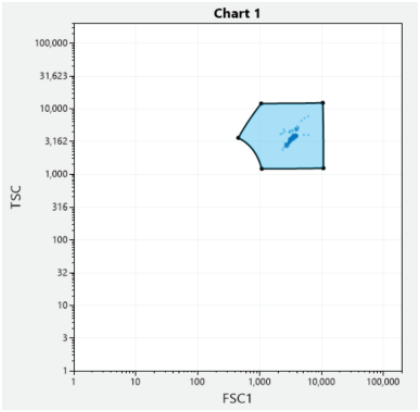




Adding Vertices to Polygon Gates (3-8 Vertices are supported for a gate)

- Right click on border of gate for desired new vertex and open chart menu
- Hover over Gate -> Add Vertex





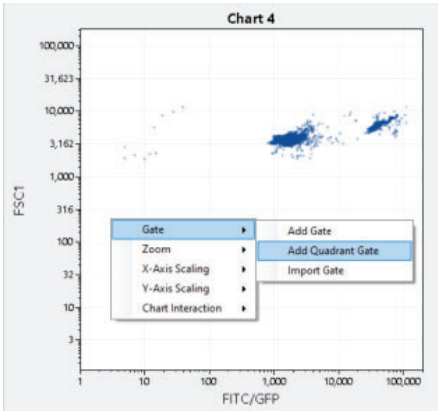
NOTE: Gate shapes that correctly function with Pala's programming will be shaded blue while gate shapes that are not allowable are shaded red and will not allow dispensing/sorting. Gates shaded red must be readjusted until they are shaded blue.

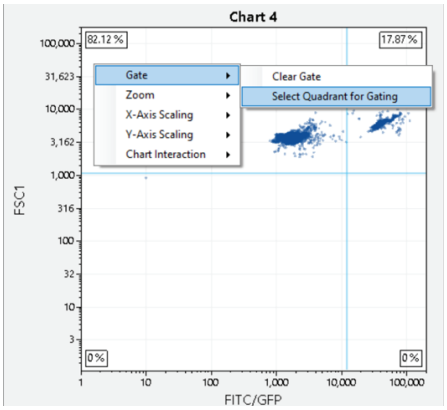
Saving Gates

- Right click on chart -> Gate -> Export Gate
- Right click on chart -> Gate -> Import Gate (option not available if gate is already present on chart)

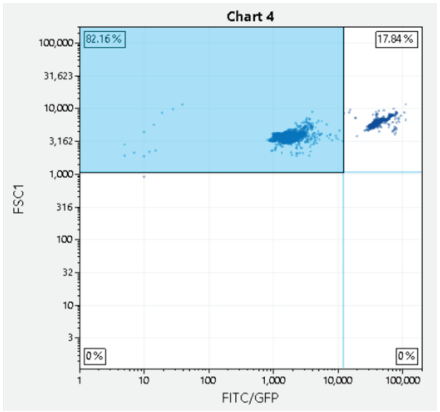
Quadrant Gates

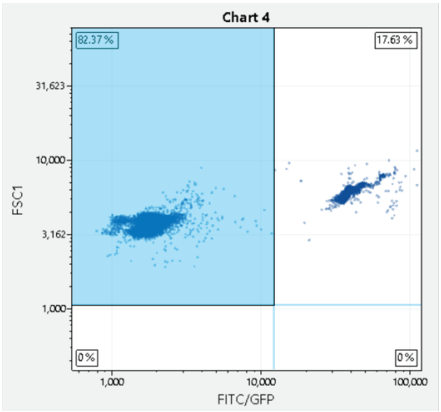
- To create Quadrant Gate, right click on any area of chart select Gate -> Add Quadrant Gate
- To adjust gate, hover over vertical or horizontal line, when double arrow appears, left click and drag lines





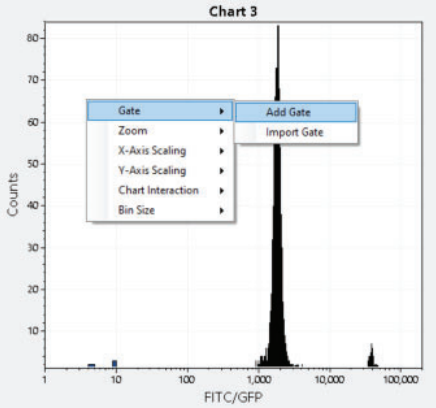
- For sorting, right click in the quadrant that is to be sorted and select Gate -> Select Quadrant for Gating
- The selected quadrant will turn blue
- Use the mouse track wheel to zoom into the region of interest

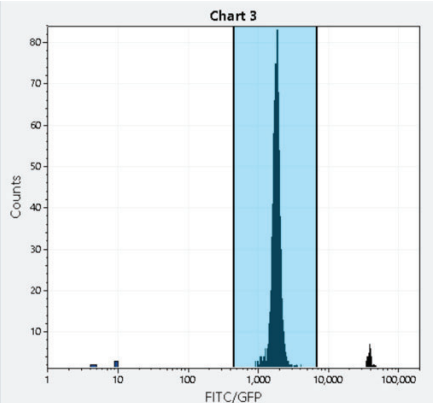




Histogram Gating

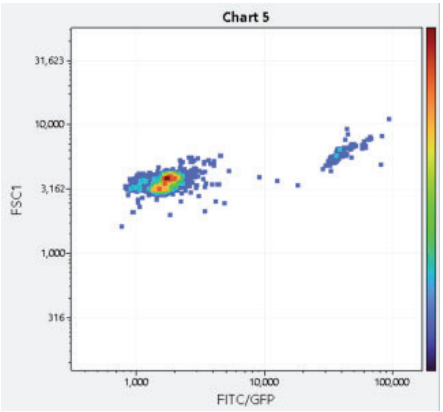
- When a histogram chart type has been selected, an interval gate can be created
- Right click to open chart menu, hover over Gate -> Add Gate
- To adjust gate, hover over gate line, when double arrow appears, left click and drag to adjust

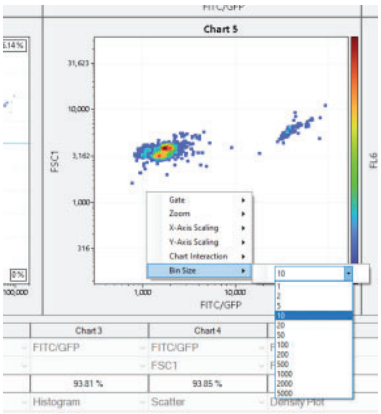




Histogram and Density Plot Bin Size

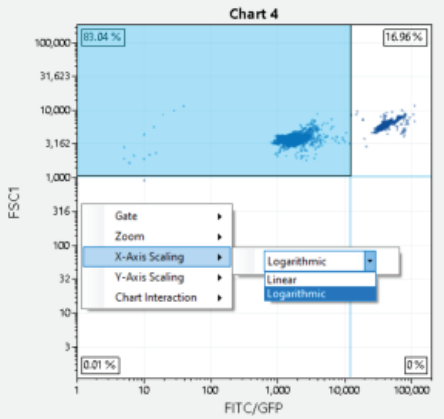
- For Histogram and Density plots, the bin size can be adjusted to help visualize the data. Increasing the bin size is a way to aggregate nearby data points into a single point of data. This can make visualization of data on the density plot and histogram more effective in certain applications. A bin size of 1 represents not binning the data.
- To adjust bin size, right click on any area of chart, hover over Bin Size -> select a bin value of 1-5000.





Log/Linear Scaling and Chart Interaction.

- Right click on chart to open chart menu
- Data plotted along the X and Y Axis can be set as Linear or Logarithmic
- Under Chart Interaction, Zoom, Pan, Scroll Zoom and Click Drag Zoom can be enabled or disabled.



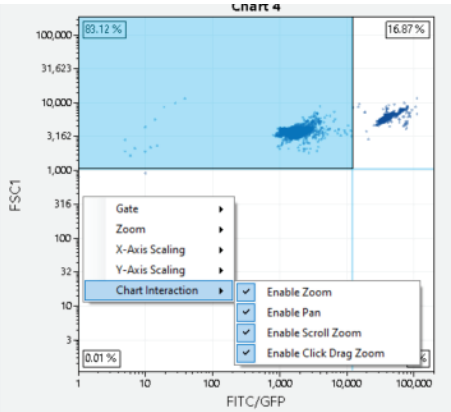


Chart Navigation Commands

Zoom

- Scroll into/out of data with mouse scroll wheel
- Right click, hold and drag to left/right (in/out on x-axis), up/down (in/out on y-axis), or diagonal up/down (in/out on both axes
- Holding Shift and dragging along y-axis locks the x-axis
- Holding Ctrl and dragging along x-axis locks the y-axis

Pan across chart while zoomed

- Left click, hold and drag across chart

Zoom to fit data

- Click scroll wheel on chart
- Or right click anywhere on chart, hover over Zoom -> Zoom to fit data

Zoom to selection

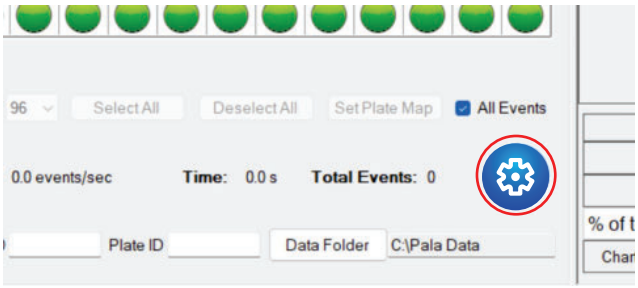
- Click scroll wheel, hold and drag a rectangle around desired area

Zoom to axis limits

- Right click anywhere on chart, hover over Zoom -> Zoom to axis limits

System Control Menu

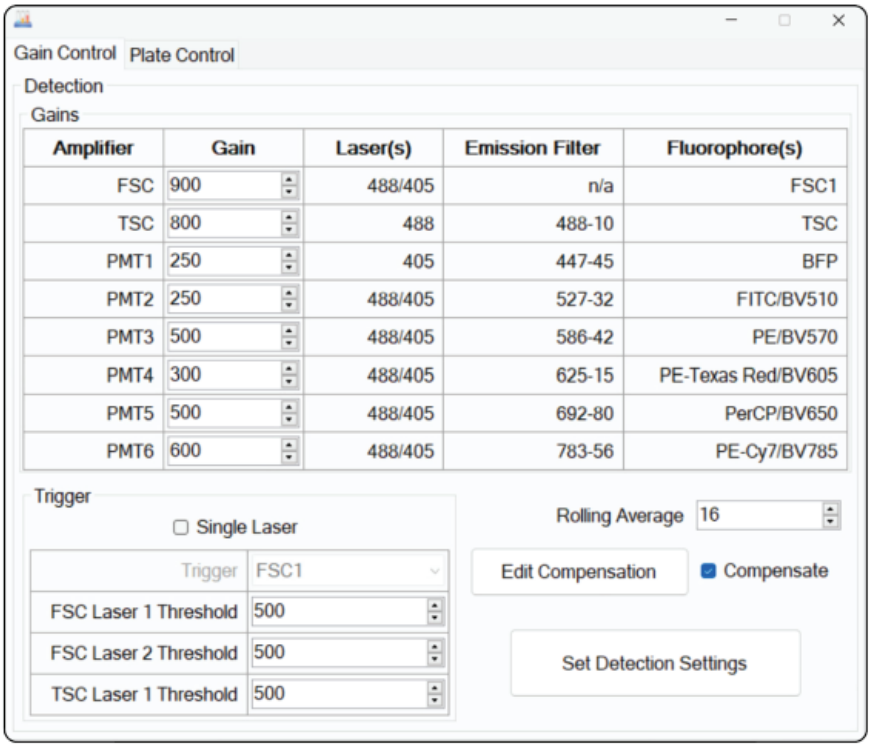
The system control menu can be accessed by clicking on the System Control icon on the main screen. The Menu will provide access to two tabs: The Gain Control and the Plate Control.



Gains Control Tab

The Pala utilizes two photodiodes for the FSC and TSC detectors and depending upon the model, has 5 (488/561 Pala) or 6 (405/488 Pala) Photomultiplier Tubes (PMTs) for the fluorescent channels. By adjusting the gains of the detectors, the signal intensity of the populations can be increased or decreased. The trigger

Once adjusted, select the “Set Detection Settings” to accept the changes.



Edit Compensation

The Compensation ban be edited by running the stained and unstained control samples and manually adjusting the compensation table accordingly so that the cell populations are properly displayed on the scatter plot.

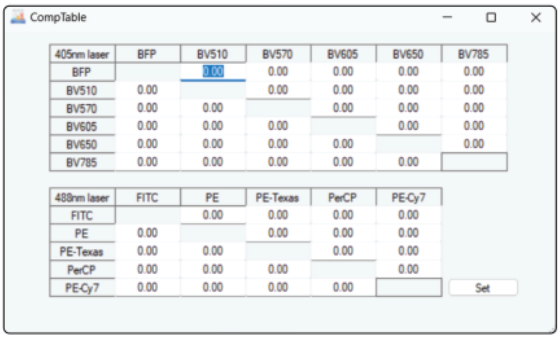


Plate Control Tab

The well to well spacing on microtiter plates can vary from manufacturer to manufacturer and format type. If the spacing needs to be adjusted, the A1 position and Jog Distance can be adjusted to ensure that the cell/droplet is accurately dispensed into the center of the well. Please note that the positioning is much more critical in the 384 well plate type since the distance between the wells and size of the well is smaller. To set the correct spacing, see **Chapter 8: Appendix**

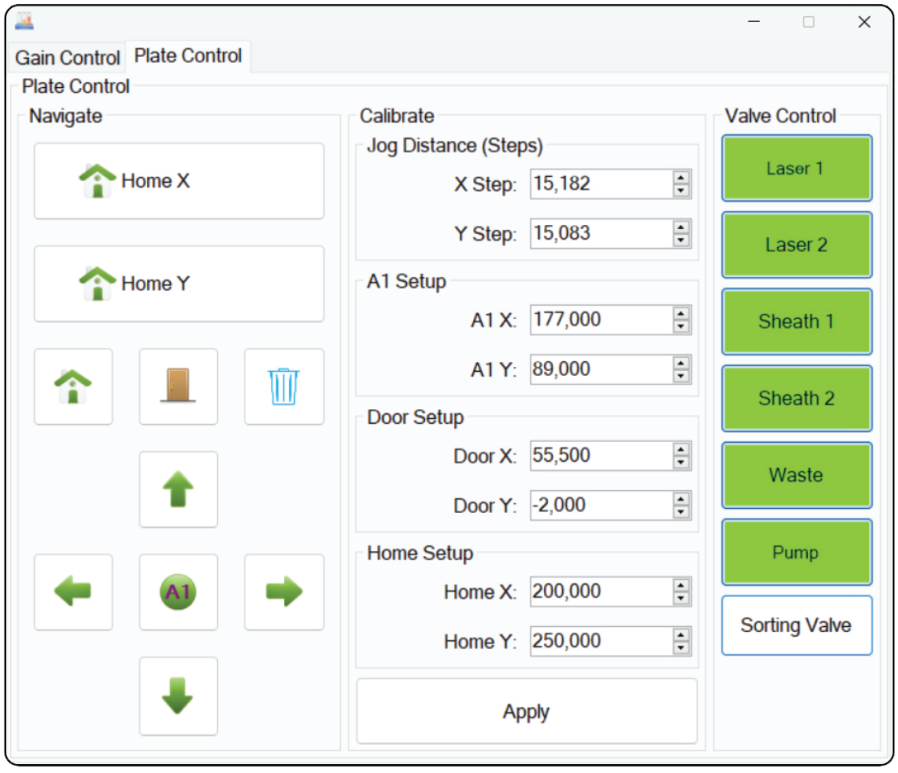
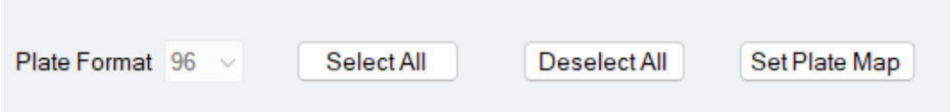
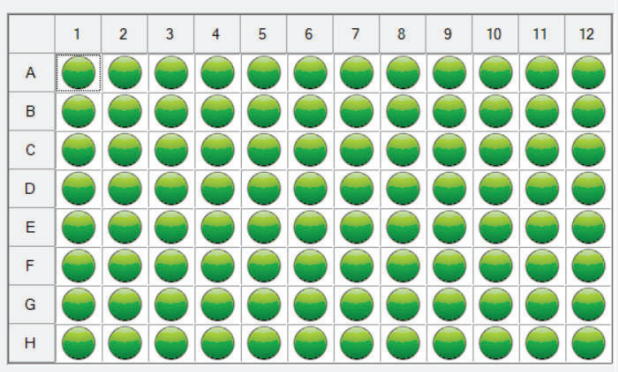
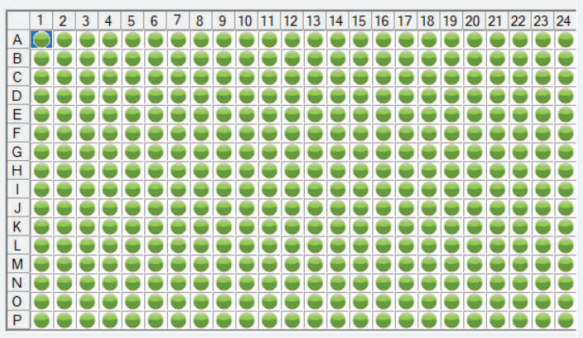


Plate Options

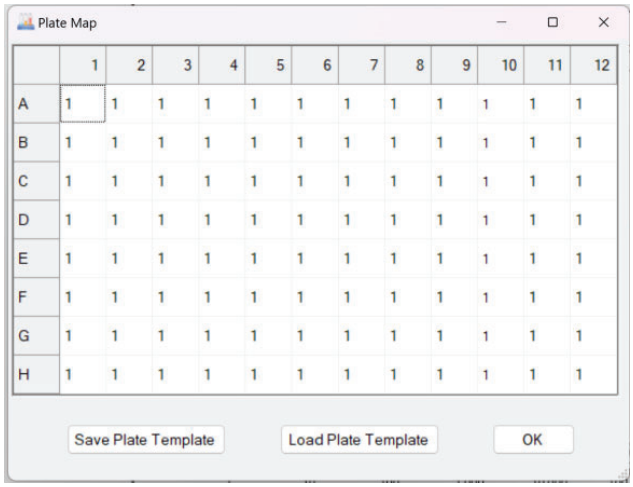
96 or 384 well plate formats can be selected under the Plate Format dropdown option.



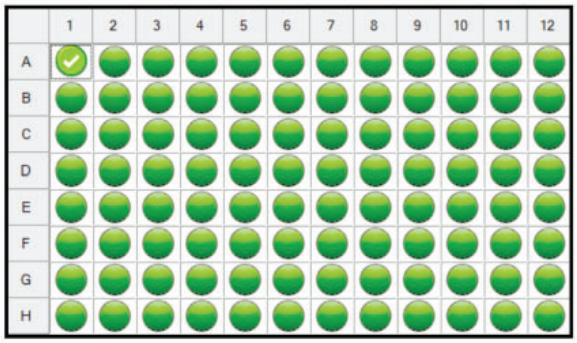




The number of cells that are dispensed into the well of a plate can also be adjusted. The Plate Map can be saved and also a template loaded from a CSV file. Please note that 1 microliter volume is dispensed with each cell so the total number of cells per well will be dependent upon the plate format and manufacturer.

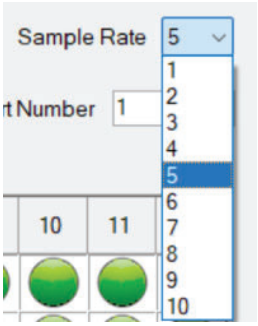


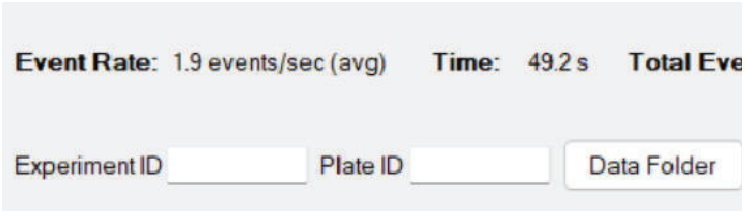
The user has the ability to select all of the wells for dispensing by clicking on the "Select All" button. Individual wells can be selected by individually clicking on the well or clicking and dragging the mouse to select a portion of the plate and then clicking any of the selected wells to select those wells.



Sample Rate

The sample rate has preset values that range from 1 -10 with 1 being the slowest and 10 being the fastest. The sample rate should be adjusted to ensure that the event rate is 1-2 cells per second in Single Cell Mode and less than 50 cells per second in Bulk Mode.





Chapter 4:

Single Cell Sorting

Chapter Overview

- Overview of Single Cell Sorting
- Sheath Buffer for Single Cell Dispensing
- Setting Up the Pala for Single Cell Dispensing
- Analysis of Cells for Single Cell Sorting
- Gating Cell Populations
- Sorting of Single Cells into Microtiterplate
- Shutting Down the Pala

Overview of Single Cell Sorting

This section provides a general overview for single cell dispensing into microtiter plates. Single cell dispensing may be performed in dual or single laser mode depending on the application. Single cell dispensing is designed to deposit single cells into wells of 96 or 384 well plates. All plates should be tested for dispensing accuracy and adjusted in the System Control Menu prior to use **(Chapter 8: Appendix)**. Please follow cell preparation guidelines **(Chapter 6: Experimental Optimization)** and optimize specific cell preparation procedures to enable single cell suspensions at the concentration recommended in this manual. It is advised that the following steps be performed immediately after cells, plates and materials have been prepared.

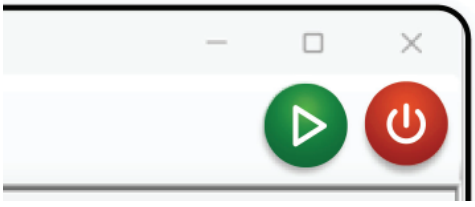
Sheath Buffer for Single Cell Dispensing

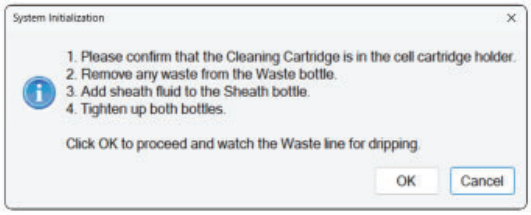
One key advantage of the Pala cell sorter is the gentle environment cells are subjected to by the instrument. Under single cell dispensing mode, cells have a very short contact time with the instrument and sheath buffer. Optimization of sheath buffer may be necessary for users to achieve the best outgrowth for their specific cell type.

Sheath buffers should be free of particulate debris such as salt crystals, protein aggregates, or other matter that may clog fluidic lines and add to optical noise. Non-aqueous solvents or solutions that do not possess similar viscosity to a balanced salt solution (i.e., 1x PBS) should be avoided. Due to the short contact time between the sheath buffer and the cell sample, it has been observed that many cell types can tolerate Cell Culture Grade Water for sheath buffer. It is advised to determine the sensitivity of the desired cell type to determine the best conditions for outgrowth. Cell Culture Grade Water will decrease wear and tear on the instrument and decrease the potential for instrument clogs. All sheath fluids must be thoroughly cleaned from the system, utilizing the Shutdown function, between all uses to prevent salt crystallization or clogs within the instrument's fluidic lines.

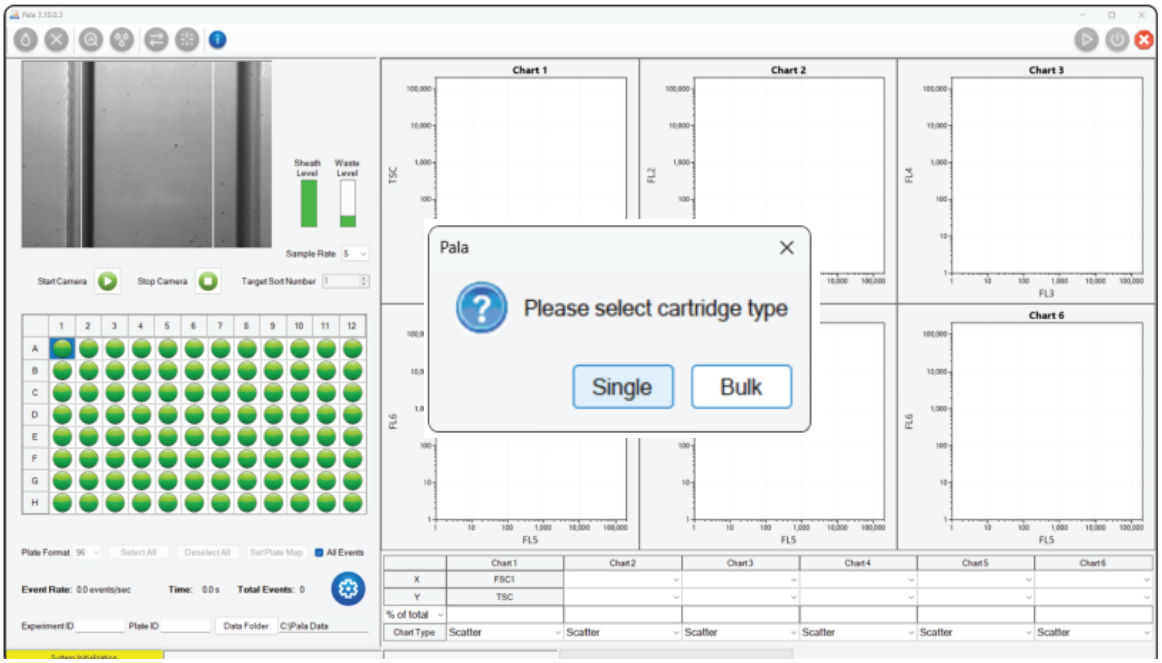
Setting Up the Pala for Single Cell Dispensing

To get started, click on the "Green Arrow" icon in the upper right hand corner to initialize the system. Proper performance requires that the system maintain stable pressures so it is critical to ensure that the lids on the bottles are properly threaded and gently tightened. Additionally, avoid bending the tubing on the bottle caps as this may result in crimps that can decrease the instrument's performance. Initialization will take about 5 minutes to complete and use 20 mL of sheath buffer.

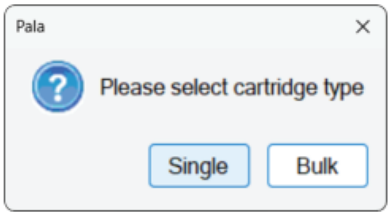


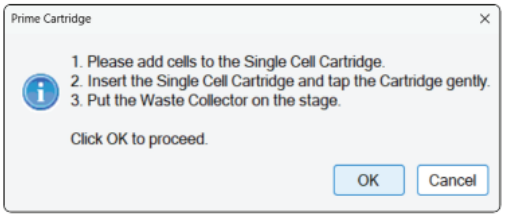


After initialization, select **"Single"** from the two cartridge type options.

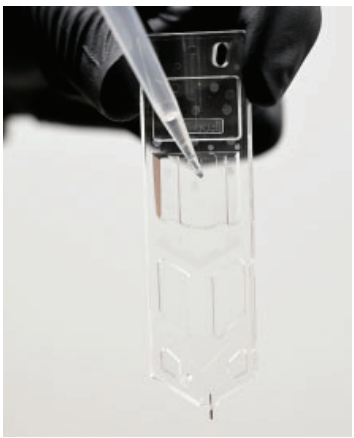


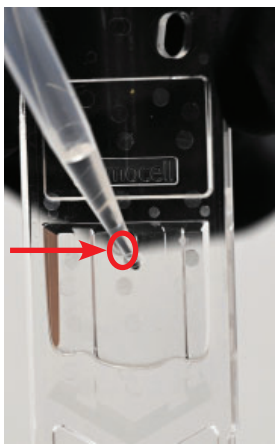
Follow the prompt on the screen.



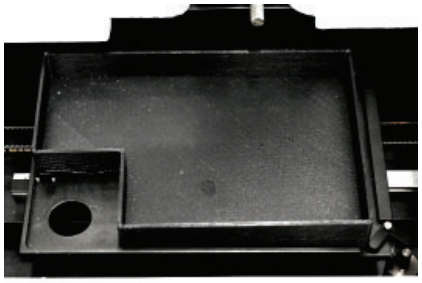


Load cell suspensions of up to 600 microliters into the small port in the middle of the single cell cartridge and gently tap the cartridge to dislodge any bubbles that may have formed in or near the channel. Hold the cartridge on the edges avoiding contact with the face of the cartridge. When inserting the cartridge into the cartridge holder, insert at the top right corner of the cartridge with the hole towards the back of the instrument using the cartridge orientation label as a guide.

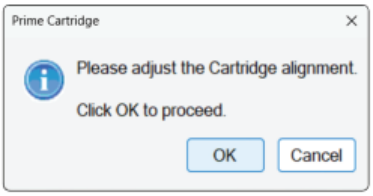




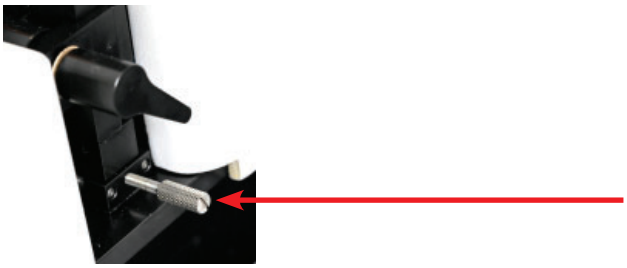
Place the Waste Collector on the stage and Click OK to proceed.



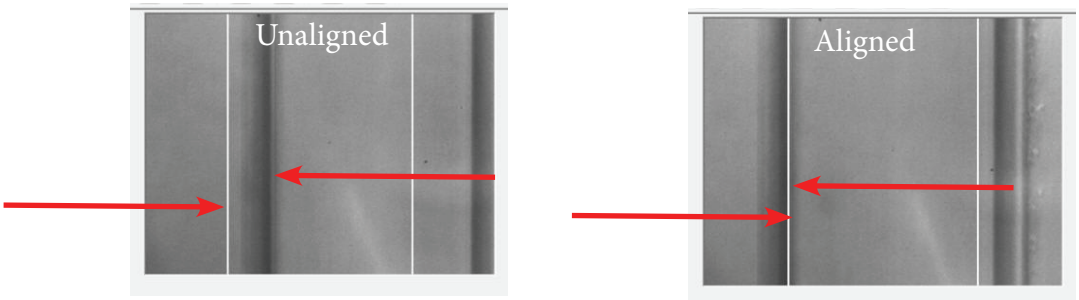
The black lever will automatically rotate clockwise to create a seal between the cartridge and the fluidic lines attached to the cartridge holder. The vacuum pump on the instrument will engage and prime the cartridge using sheath buffer. After priming, a pop-up screen will appear to prompt the user to align the cartridge.



Cartridges loaded into the cartridge holder may need a slight alignment so that the lasers are properly focused onto the cell channel. The cartridge holder has a stainless steel positioner knob that moves the cartridge from side to side so that the lasers may be focused onto center of the channel. At this point, a live camera image of the channel will appear.



Align the image so that the dark line of the channel aligns to the left white line in the image. Once aligned, click OK to proceed. If the camera image is dark, the cartridge holder may need to be dried with a sterile cotton swab. See **Chapter 7: FAQ and Troubleshooting** for additional instructions. .



During the cartridge priming process a small amount of sheath buffer will be dispensed through the nozzle and onto the waste collection tray. This is a normal process for clearing air bubbles from the cartridge to ensure proper dispensing performance.

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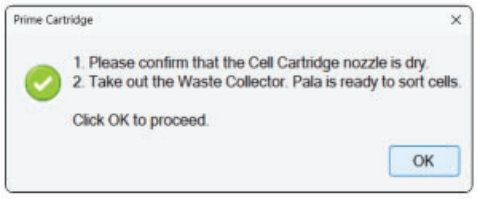
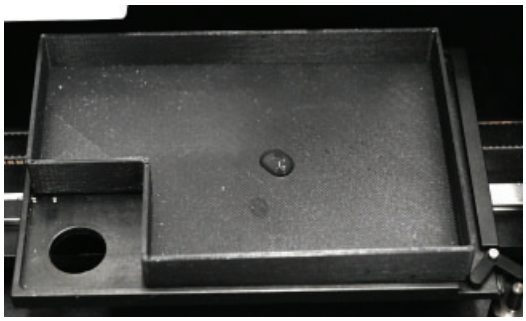
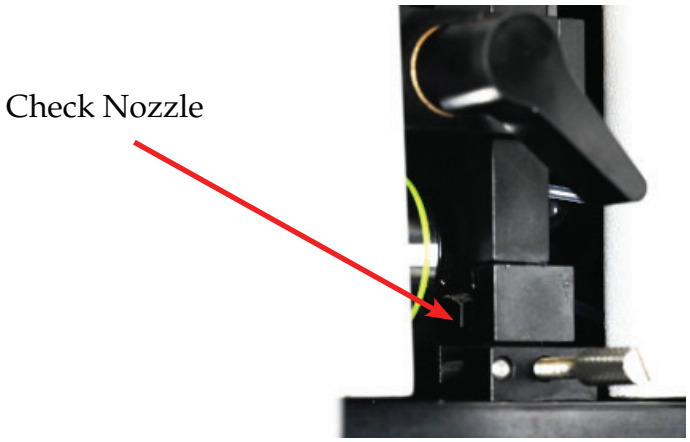
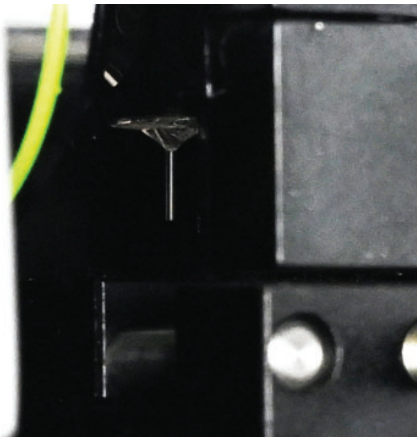




Confirm that the cartridge nozzle is not dripping (watch for ~10-20 seconds). We have included a small flashlight to better visualize if the nozzle is dripping. A dripping nozzle will adversely affect single cell deposition results. Please see **Chapter 7: FAQ and Troubleshooting** a dripping nozzle.

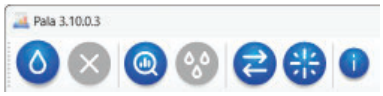




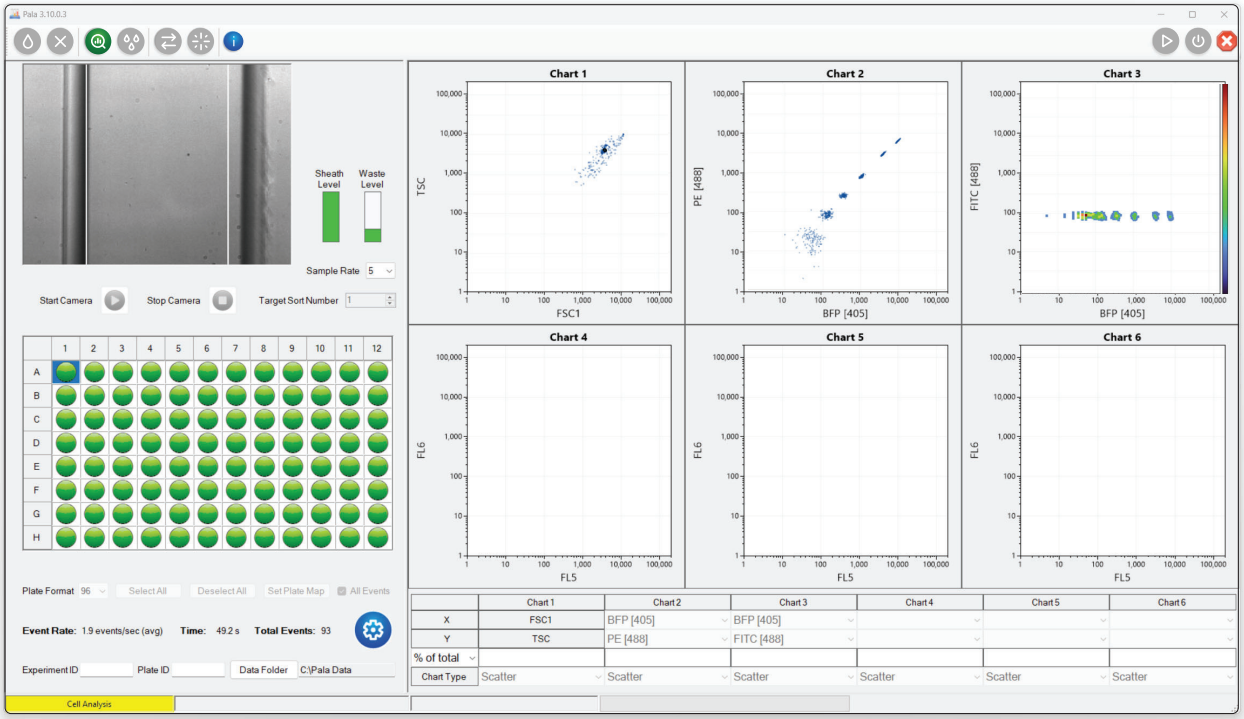
Analysis of Cells for Single Cell Sorting

Prior to dispensing, cells must be analyzed with user selected charts and at least one gate must be drawn to direct the software to dispense cells. First, select the appropriate channels from the dropdown for each chart axis. As an example, Rainbow Beads (wide spectrum for fluorescence) with 6 different populations with varying intensity have been used for the following instructions. Instructions for optimum single cell sorting are discussed in **Chapter 6: Experimental Optimization**.

	Chart 1	Chart 2	Chart 3	Chart 4	Chart 5	Chart 6
X	FSC1	FITC/GFP	FITC/GFP	FITC/GFP		
Y	TSC	PE/DsRed	FSC1			
% of total	89.56 %	57.95 %				
Chart Type	Scatter	Scatter	Density Plot	Histogram	Scatter	Scatter

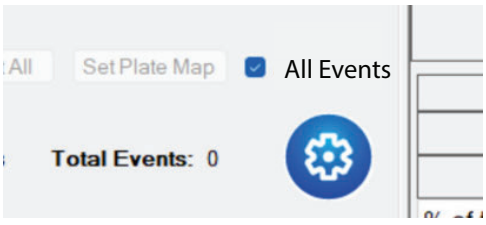


Once the cartridge has been aligned and charts selected, click on the "Cell Analysis" icon to start the analysis. Once Analysis has started, the icon tray will disable the other icons from being selected and turn the "Analyze" icon green. To stop the analysis at any time, click the "Cell Analysis" again. Once analysis has started, the detected events will be plotted on the selected charts.

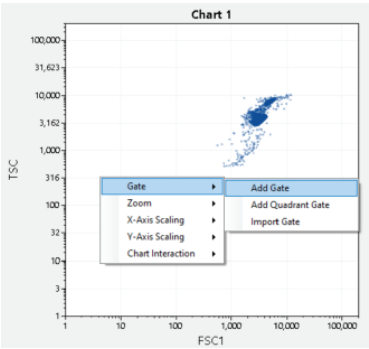


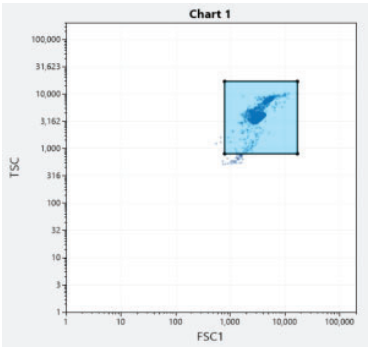
Gating Cell Populations

For Chart 1, the default channels are always FSC1 and TSC (Forward Scatter vs Total Scatter). When "All Events" is checked (by default), all detected cells will be displayed on all the open charts. If "All Events" is unchecked by the user, the gating populations will cascade from Chart 1 to Chart 6. In other words, only the events gated in a chart will appear for gating in subsequent charts. For example, if a population of fluorescent cells are gated in Chart 2, then only those cells will be displayed on Chart 3 and only cells gated in Chart 3 will be displayed for gating in Chart 4 and so forth.

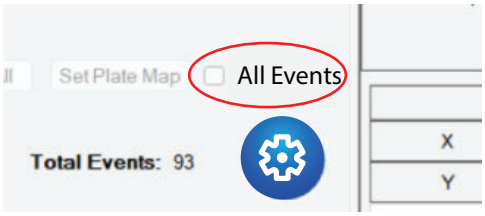


To draw a gate, right click on any of the charts and select “Add Gate”. Additional instructions for creating, moving, sizing or editing a gate are located in **Chapter 3: Software Commands and Interface**.



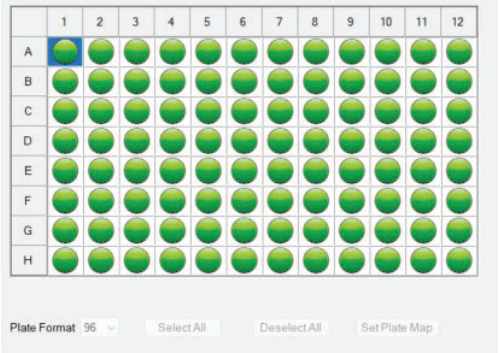


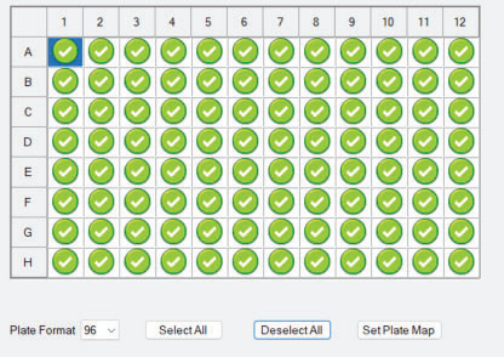
In the example below, “All Events”were unchecked and gates were created for Charts 1 and 2 and Charts 3 and 4 do not display the 2 dimmer bead populations.



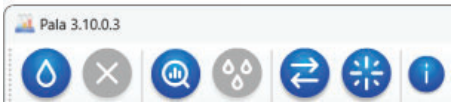
Sorting of Single Cells into Microtiterplate

The user must ensure "Cell Analysis" is stopped by clicking on the icon to proceed with dispensing. First, select the appropriate Plate Format (96 or 384). Next, wells on the plate map must selected. Either all wells may be selected with "Select All" or a portion of wells can selected by clicking in the white space around the circle, dragging a selection, and clicking inside a circle of a well in the selection. Individual wells may be selected by left clicking in the green circle of any well. Selected wells display a white check mark. Please note that more than 1 cell can be dispensed into a well by clicking on "Set Plate Map" and either typing or loading a CSV file specifying the number of cells/well to be dispensed.

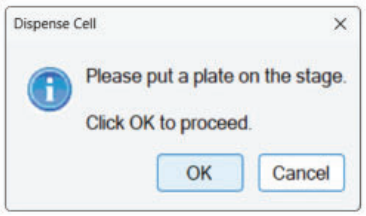


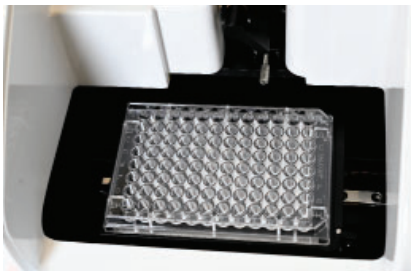


To initiate dispensing, click on the droplet icon .



Place a 96 well or 384 well plate with the appropriate media into the plate holder and click OK to proceed.

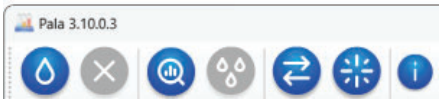


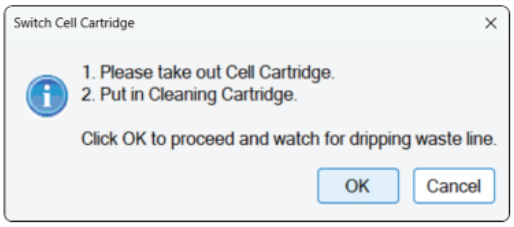


As the cells are dispensed, the well will receive a red check mark. Dispensing can be aborted at any time by clicking on the cancel icon .

Once dispensing is completed, a prompt will indicate that the plate can be removed. Additional plates can be dispensed if desired by clicking on the droplet icon again.

If more sample, a different sample, or different cartridge is needed, a cartridge switch can be performed by selecting the "Switch" icon

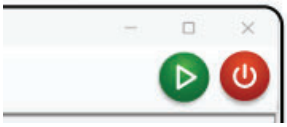


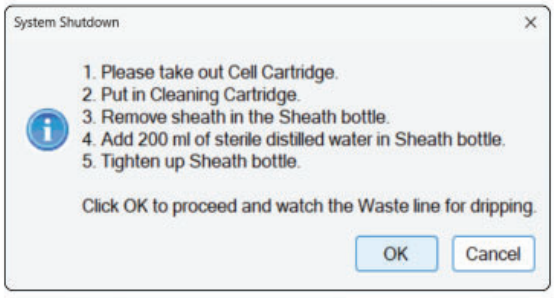


Shutting Down the Pala

Instrument shutdown must be performed at the end of each day when using the instrument even if only sterile water was used as sheath buffer. This process will clear out cells and media from the waste fluidics and prompt the user to clean the waste bottle. Shutdown is also necessary to close the Pala software.

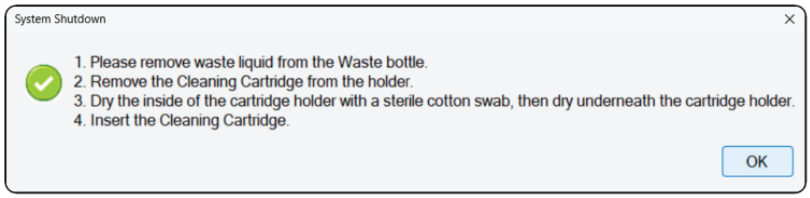
When shutting down the Pala, click on the "Shutdown" icon in the upper right hand corner of the software.



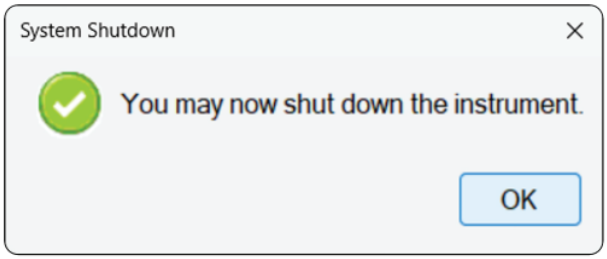


Follow the instructions on the screen ensuring that sterile ddH20 is used in the sheath bottle. Failure to properly perform the shutdown procedure will increase the likelihood of catastrophic blockage that will require service. Shutdown takes 5 minutes to complete.

After the initial shutdown has completed, follow the second set of instructions.



Once the System Shutdown has completed, the instrument can be turned off and the software can be exited.



Chapter 5:

Bulk Cell Sorting

Chapter Overview

- Overview of Bulk Sorting Cells
- Analyzing Cells for Bulk Cell Sorting
- Gating Cell Populations
- Sorting Cells into a Microcentrifuge Tube
- Shutting Down the Pala

Overview of Bulk Sorting Cells

This section will provide a general overview of bulk sorting. The bulk sorting can be performed under dual or single laser mode depending on the application.

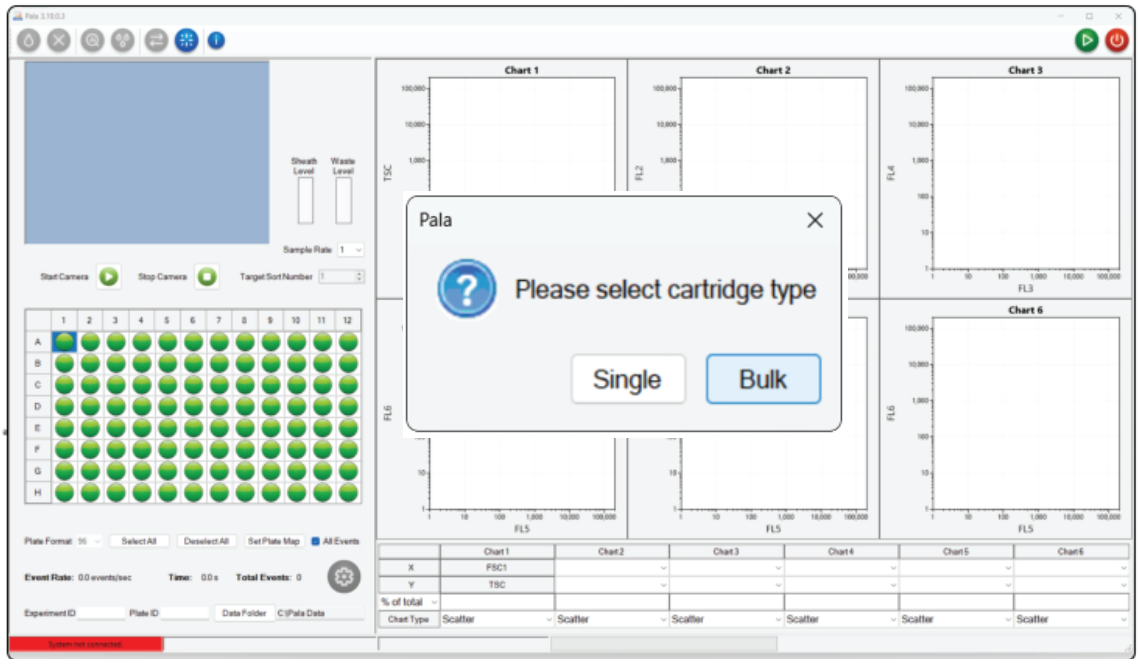
The Pala is capable of sorting a desired number of cells into a micro-centrifuge tube. Please follow cell preparation guidelines in **Chapter 6: Experimental Optimization** to ensure single cell suspensions at the appropriate concentrations for the Pala.

Unlike Single Cell Sorting, ddH20 should not be used as the sheath buffer. Since cells will be sorted directly into a tube and not a microtiter well filled with media, the sheath fluid should contain a buffered and pH balanced solution such as Phosphate Buffered Saline (1X PBS). The sheath buffer should be free of particulate debris such as salt crystals, protein aggregates, or other matter that may clog fluidic lines and add to optical noise. Non-aqueous solvents or solutions that do not possess similar viscosity to a balanced solution should be avoided.

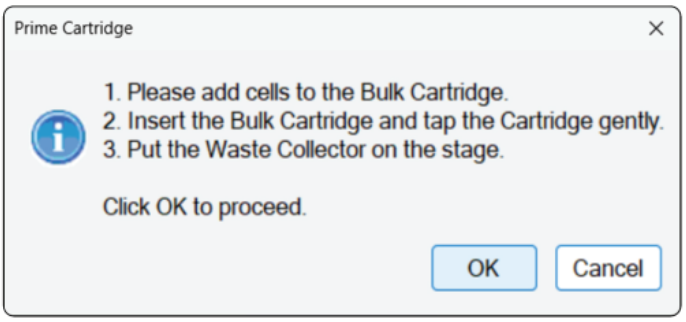
Setting Up the Pala for a Bulk Sort

To get started, first ensure that the system is properly initialized as described in Chapter 4 - Single Cell Sorting.

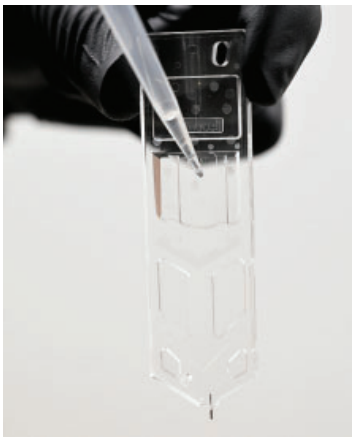
After initialization, select **"Bulk"** from the two cartridge type options.

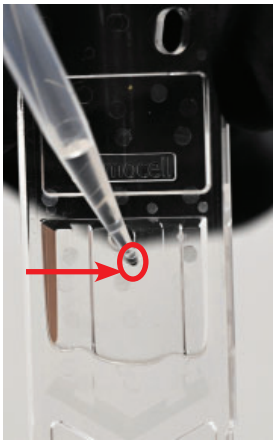


After clicking on the **"Bulk"**, the lever on the cartridge holder will automatically rotate to unlock the cartridge and prompt you to remove the cleaning cartridge.



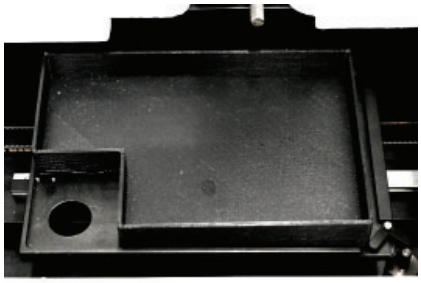
Load cell suspensions of up to 600 microliters into the small port in the middle of the bulk cartridge and gently tap the cartridge to dislodge any bubbles that may have formed in or near the channel. Hold the cartridge on the edges avoiding contact with the face of the cartridge. When inserting the cartridge into the cartridge holder, insert at the top right corner of the cartridge with the hole towards the back of the instrument using the cartridge orientation label as a guide.





Place the Waste Collector on the stage and Click OK to proceed

.



The black lever will automatically rotate clockwise to create a seal between the cartridge and the fluidic lines attached to the cartridge holder. The vacuum pump on the instrument will engage and prime the cartridge using sheath buffer. After priming , a prompt will appear prompting the user to align the cartridge.

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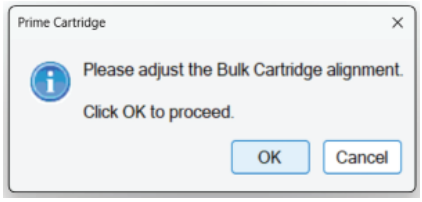
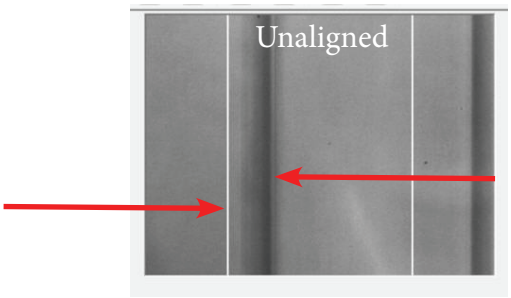
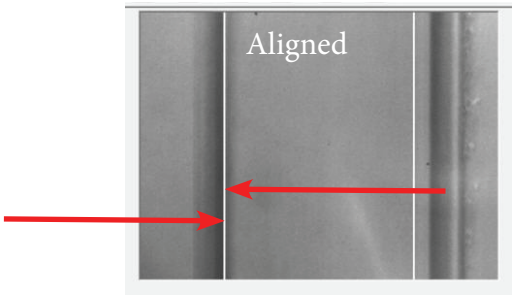


Cartridges loaded into the cartridge holder may need a slight alignment so that the lasers are properly focused onto the cell channel. The cartridge holder has a stainless steel positioner knob that moves the cartridge from side to side to enable the lasers to be focused directly onto the center of the channel. At this point, a live camera image of the channel will appear.

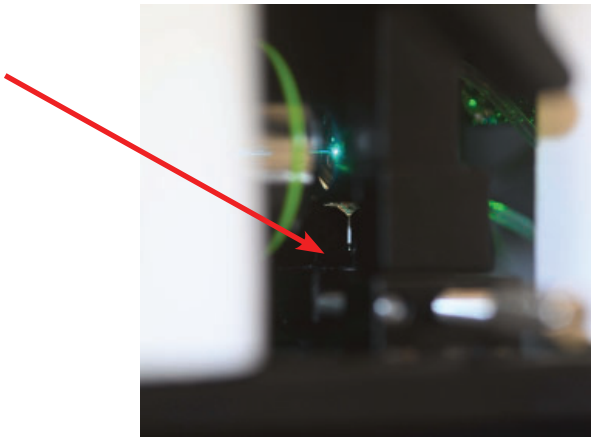
Align the image so that the dark line of the cartridge channel aligns to the left white line in the image. Once aligned, click OK to proceed. If the camera image is dark, the cartridge holder may need to be dried with a sterile cotton swab. See **Chapter 7: FAQ and Troubleshooting** for additional instructions .

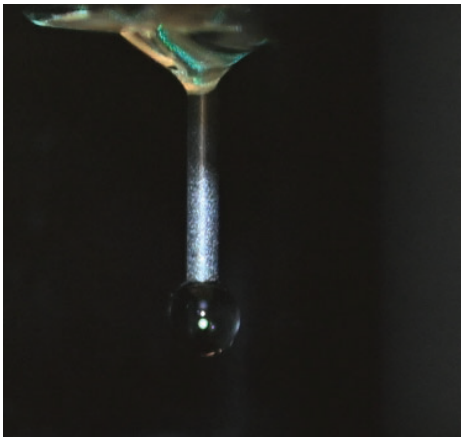




During this step, the cartridge will be primed and small amount of sheath buffer will be dispensed from the nozzle. Please note that the volume dispensed by the bulk cartridge during priming will be approximately 1/4th what is dispensed during single cell cartridge priming.

Unlike the single cell cartridge, the bulk cartridge nozzle will contain a small amount of sheath buffer that will slowly form and drip from the nozzle. This is part of the sorting design of the Pala and is normal. You will not be prompted to determine if the nozzle is dripping prior to sorting.





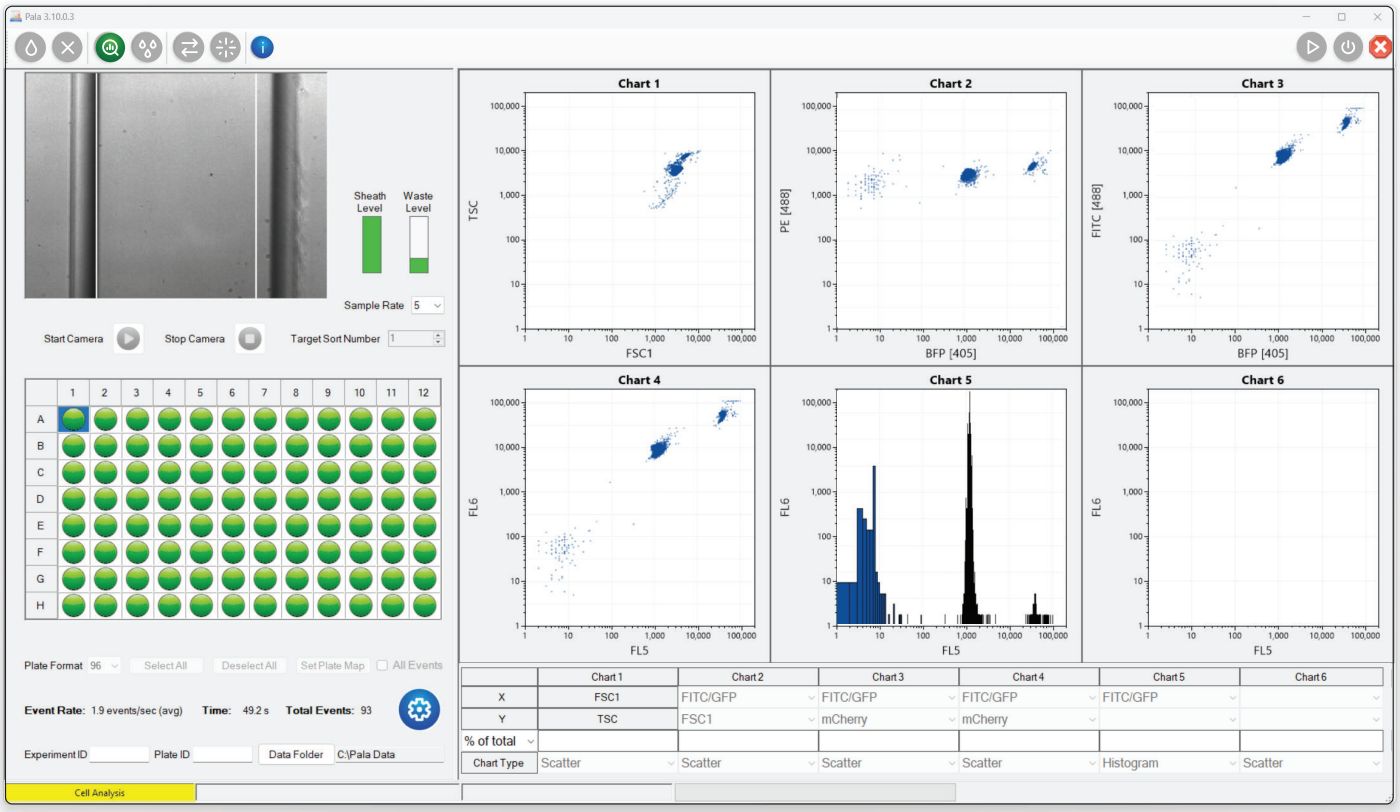
Analyzing Cells for Bulk Cell Sorting

Similar to Single Cell Sorting, the first step in Bulk Cell Sorting, is to analyze the cells on the charts and visualize the data as scatter plots or histograms. Assign the appropriate fluorescent dyes or scatter property with the chart type of scatter, histogram, or density plot. A mixture of 2 different bead populations are used to demonstrate how the Pala can increase the purity of a population. In the example below, 6 micron beads with Nile Red and 10 micron beads with FITC are mixed where the FITC population is at 20%. After bulk sorting, the purity of the FITC population can be increased up to 90%. Additional information on bulk dispensing can be found in discussed in **Chapter 6: Experimental Optimization**.

	Chart 1	Chart 2	Chart 3	Chart 4	Chart 5	Chart 6
X	FSC1	FITC/GFP	FITC/GFP	FITC/GFP	FITC/GFP	
Y	TSC	FSC1	mCherry	mCherry		
% of total	100 %	33.03 %				
Chart Type	Scatter	Scatter	Scatter	Scatter	Histogram	Scatter

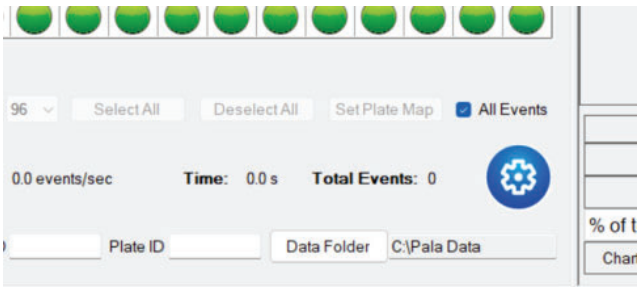
With the cell cartridge inserted, click on the "Analysis" icon Once Analysis has been clicked, the icon tray will disable the other icons from being selected and turn a green icon . Detected events will be plotted on the open charts. To stop analysis at any time, click again on the icon.

The figure below is an example of how cell populations may display.

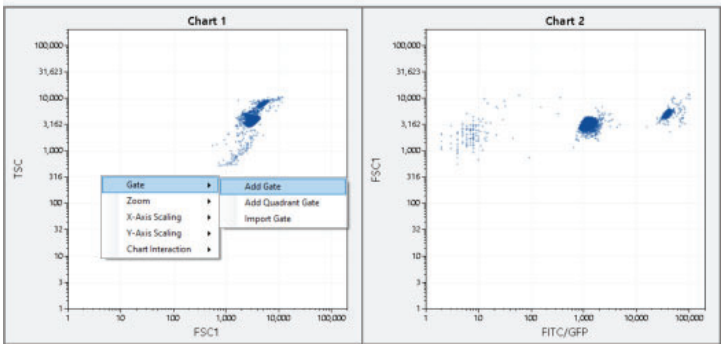


Gating Cell Populations

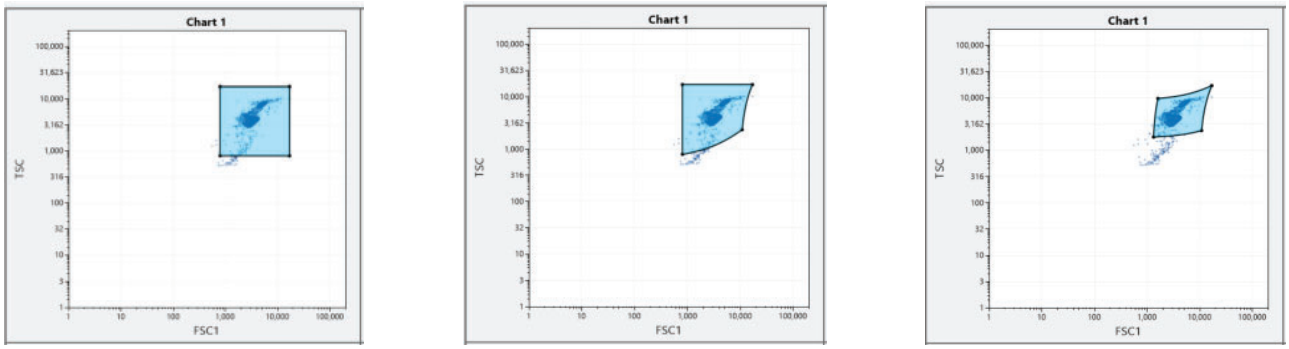
For Chart 1, the default channels are always FSC1 and TSC (Forward Scatter vs Total Scatter). When “All Events” is checked (by default), all detected cells will be displayed on all the open charts. If “All Events” is unchecked by the user, the gating populations will cascade from Chart 1 to Chart 6. In other words, only the events gated in a chart will appear for gating in subsequent charts. For example, if a population of fluorescent cells are gated in Chart 2, then only those cells will be displayed on Chart 3 and only cells gated in Chart 3 will be displayed for gating in Chart 4 and so forth.



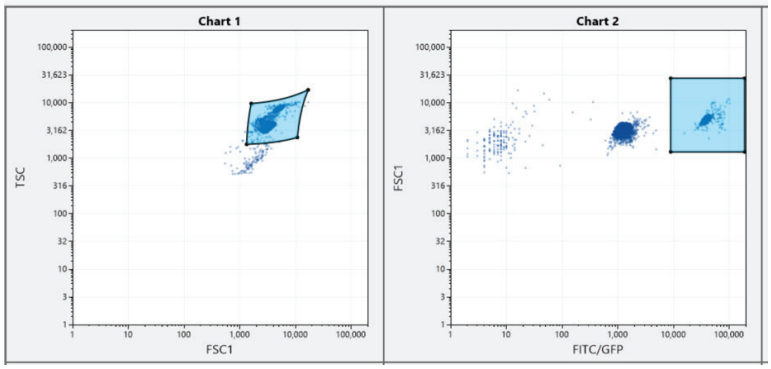
To draw a gate, right click on any of the charts and select "Add Gate". Additional instructions for creating, moving, sizing or editing a gate are located in **Chapter 3: Software Commands and Interface.** .



In the example below, a gate was added to Chart 1 (default always FSC/TSC). Adjust gate size, shape, and number of vertices.



In this example, a second gate was added to FITC/FSC1 channels plotted on Chart 2. Note that when gates are drawn on multiple charts that only cell populations that meet requirements between gates in both Charts 1 and 2 will be sorted.



Sorting Cells into a Microcentrifuge Tube

The user must ensure that analysis has been completed before proceeding to sorting. Click on the green A icon to stop analysis. Next, the user must specify the number of desired target cells to be sorted.

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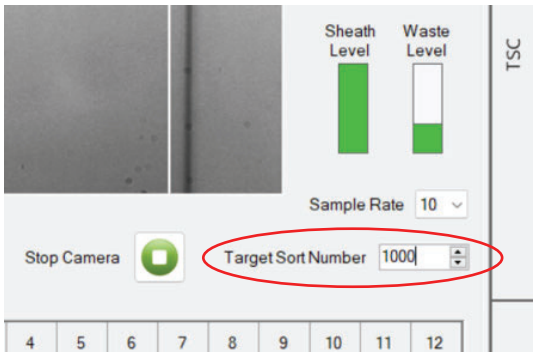
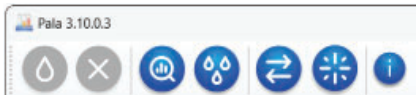
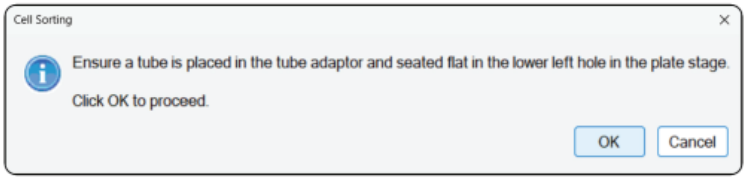
In this example, 1000 cells were set as the Target Sort Number.



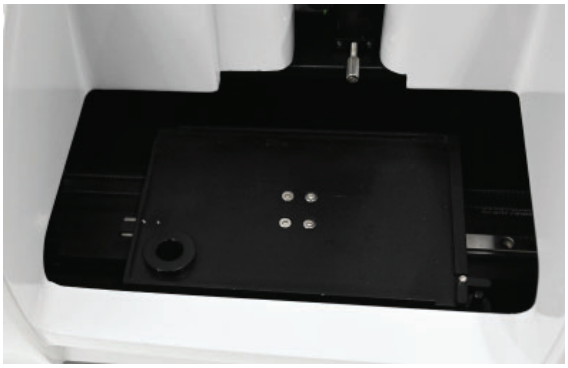
Once the Target Sort Number has been set, click on the "Bulk Dispense" icon to start the sort. The icon will turn green when sorting is in progress .

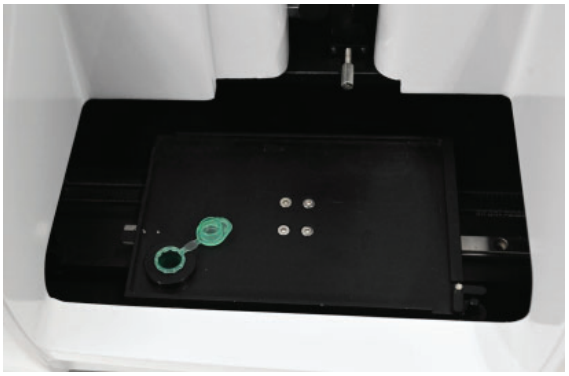


Follow the on-scren prompt and select OK to start sorting.

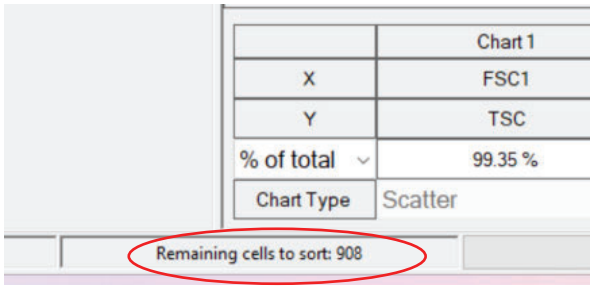


Ensure that the microcentrifuge tube adapter is used with the microcentrifuge tube in the hole located at the lower left position of the plate stage.





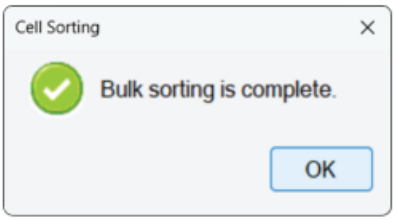
The plate stage will position the tube directly under the nozzle of the cartridge and cells will be deposited directly into the microcentrifuge tube. As the cells are being sorted, the software displays the number of remaining cells left to be sorted at the bottom software interface.



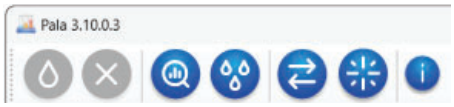
Note: A droplet will form at the tip of the nozzle and once large enough, gravity will cause the droplet to fall into the microcentrifuge tube.

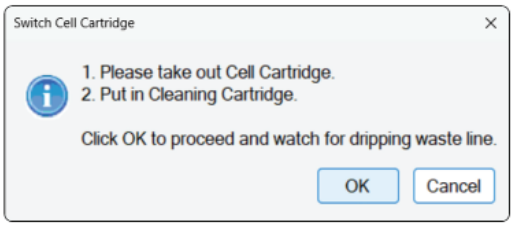
At any point during the sort, this process can be canceled by clicking on the active "Bulk Dispensing" icon .

Once the sort has completed, the plate holder will move back to the home position and the tube containing sorted cells can be removed.



Additional tubes can be collected using the same sample by simply clicking on the "Bulk Dispensing" icon again. If a different sample or cartridge is needed, a cartridge switch may be performed by selecting the "Switch" icon

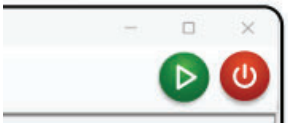


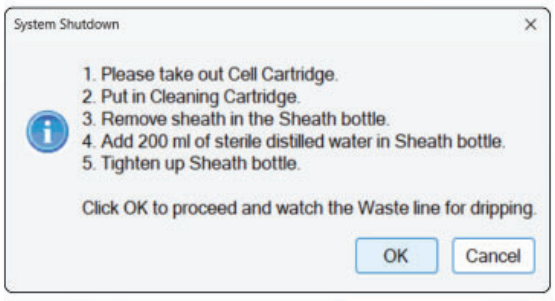


Shutting Down the Pala

After sorting is complete for the day, please perform the shutdown procedure.

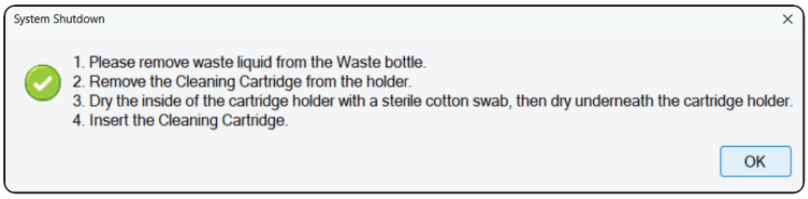
Initiate the instrument shutdown by clicking on the red square icon in the upper right hand corner of the software interface.





Follow the instructions on the screen ensuring that sterile ddH20 is used in the sheath bottle. Failure to properly perform the shutdown procedure will increase the likelihood of catastrophic blockage that will require service. Shutdown takes 5 minutes to complete.

After the initial shutdown has completed, follow the second set of instructions. Once the System Shutdown has completed, the instrument can be turned off and the software can be exited.



Once the System Shutdown has completed, the instrument can be turned off and the software can be exited. If a different sample is needed, a Switch Cartridge can be performed by selecting the S Icon or if completed a Shut Down can be performed by selected the Red Square icon.

If a different sample is needed, a Switch Cartridge can be performed by selecting the S Icon or if completed a Shut Down can be performed by selected the Red Square.

Chapter 6:

Experimental Optimization

Chapter Overview

- Fluorophore Selection
- Bio-Techne Academy
- Performance Testing Using Bead Controls

Fluorophore Selection

Pala is configured with two excitation lasers in two different configurations: with 488 & 561 nm or 405 & 488 nm lasers. Additionally, Pala comes with either 5 or 6 detection modules with the following filters:

Filter	405/488 nm	488/561 nm
447/45	√	
527/30	√	√
586/42	√	√
625/15	√	√
692/80	√	√
783/56	√	√

A fluorescent channel refers to the specific excitation laser/filter combination. The 405/488 Pala has 11 fluorescent detection channels while the 488/561 Pala has 9 fluorescent detection channels. The channels are currently named according to a well-known fluorophore with excitation/emission spectra that match the corresponding fluorescent channel characteristics

488/561 Pala

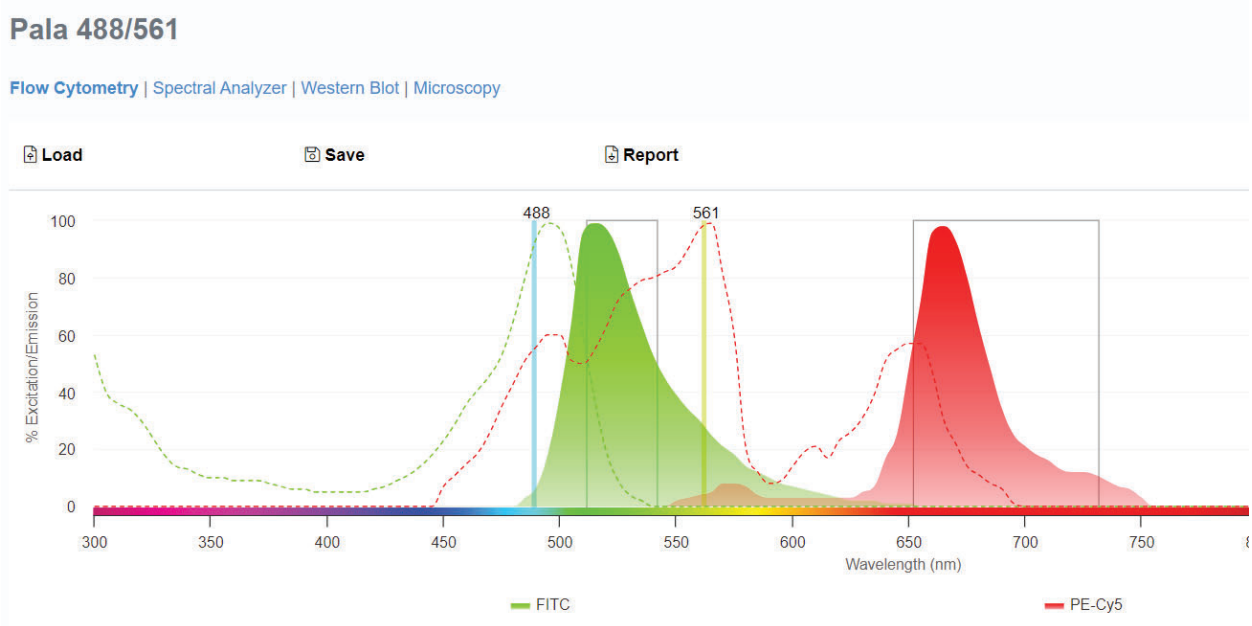
PMT	Filter	488 Channel Name	561 Channel Name
PMT 1	527/30	FITC/GFP	X
PMT 2	586/42	Nile Red	PE/DsRed
PMT 3	625/15	488/625	mCherry
PMT 4	692/80	PerCP/PI	PE-CY5
PMT 5	783/56	488/783	PE-CY7

405/488 Pala

PMT	Filter	405 Channel Name	488 Channel Name
PMT 1	447/45	BFP	
PMT 2	527/30	BV510	FITC/GFP
PMT 3	586/42	BV 570	PE/DsRed
PMT 4	625/15	BV 605	PE-Texas Red
PMT 5	692/80	BV 650	PerCP, PI
PMT 6	783/56	BVf 785	PE-Cy7

Utilize the Bio-techne Spectra Viewer to optimize fluorophore selection and Pala channel selection. **https://www.bio-techne.com/resources/spectra-viewer.**

Using this viewer, the fluorescent molecule of choice can be mapped to the corresponding channel on the Pala. In the following example, the Spectra Viewer is used to visualize 4 popular fluorescent molecules: GFP, TRITC, PE-Cy5 and PE-Cy7.



In this example where 2 dyes are used with 2 lasers, the FITC will be optimally excited by the 488 laser and detected with the 527/32 filter while the PE-Cy5 will be optimally excited by the 561 laser and detected by the 692/80 filter.

If designing an experiment where multiple fluorescent markers will be used, it is best to take into consideration the following characteristics of a fluorescent marker:

- Excitation and emission profile
- Relative brightness
- Quantum yield
- Emission overlap
- Stability of antibody-fluorophore conjugate
- Reproducibility of antibody conjugation

Follow some general guidelines in designing your experiment such as:

- Ensure that the fluorophores match the optical properties of the Pala.
- Always consider the excitation and emission maximums as well as the Stokes shift in designing your experiment with multiple fluorescent markers
- Choose the brightest set of fluorophores for your experiment and optimize brighter fluorophores to be paired with lower expression targets or weaker affinity antibodies
- Minimize spectral overlap as much as possible

- Consider photostability of fluorophore before and after fixation if necessary
- Flow Cytometry Troubleshooting Guide- Bio-Techne: **https://www.bio-techne.com/applications/flow-cytometry/flow-cytometry-troubleshooting-guide**

Bio-Techne Academy

We have created online courses for the Pala platform and users can sign up with the link below.

https://academy.bio-techne.com/learn/signin

Single Cell Dissociation

In order to maximize the effectiveness of the Pala for single cell dispensing and bulk sorting applications, it is crucial to dissociate cell cultures into single cell solutions. Utilize gentle enzymatic methods for obtaining single cells such as 0.25% Trypsin or 1x Accutase or a reagent optimized for your cell type. Additionally, clumps will clog the cell cartridge and reduce the capacity of the Pala to sort cells. Filter cells through a cell strainer that removes large clumps of cells (>40 um) just prior to loading into the Pala Cartridge.

Cell Counting & Concentration

Count cells using an automated counter or a hemacytometer that can exclude dead cells and debris. Resuspend cells in 0.2 micron filtered media or buffer of choice. Utilize the following table for recommended cell concentrations:

Sorting Mode	Cell Concentration Range	Recommended Event Rate or Sample Rate
Single Cell Dispensing	5,000-10,000 cells/ml	1-2 cells/second
Bulk Sorting	100,000-500,000 cells/ml	<50 cells/second
Rare Cell Enrichment	1M-100M cells/ml	Sample Rate = 1

Maintain Cell Health

- Use the following tips to maintain optimal cell health during sorting:
- Plan ahead - prepare reagents, plates and perform instrument set up (i.e. sterilization, preparing sheath buffer, etc…) before harvesting cells from culture. Minimize the time cells are outside of the incubator.
- Consider supplementing collection tubes/wells or cell dilution buffer with FBS or using 0.2 micron conditioned media.
- Never single cell dispense into an empty plate, always prepare the plate pre-filled with media.
- Test the viability of your cell line pre- and post-sorting to understand what to expect and make optimizations to your process.
- Although cell culture grade water has been successfully used for single cell dispensing (due to the very short contact time between sheath buffer and cells) it is not recommended for use in bulk sorting since cells are in contact with sheath buffer for a longer period. For bulk sorting, use 1X PBS or a suitable balanced salt solution for sheath buffer.

Performance Testing Using Bead Controls

Three types of control beads are used for testing Pala performance: Linearity 6 peak 6 micron (RCP001-60-10), Accuracy 10 micron (URFP001-100-10), and the Bulk Sorting Mix 6 micron Nile Red/10 micron Rainbow 80/20 mix (URFP-1006056-10). The following are three protocols for testing Pala performance.

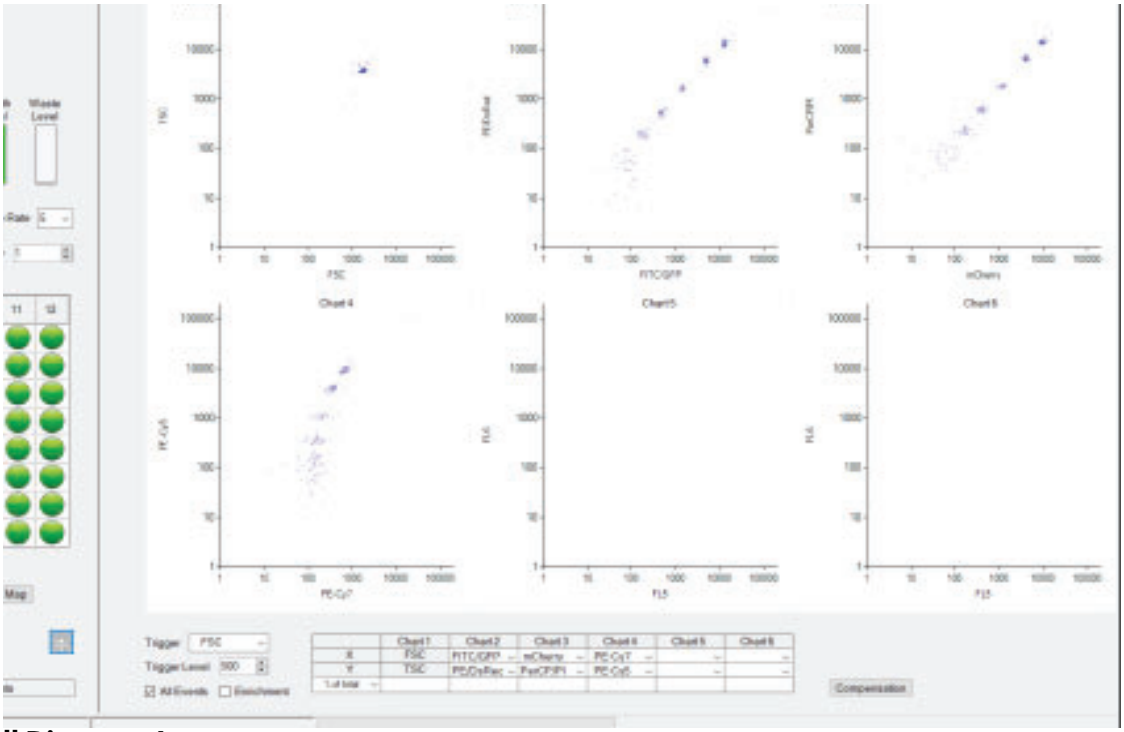
Testing laser alignment and PMT function

Prepare a diluted aliquot of the Linearity 6 peak 6 micron beads (RCP001-60-10).

1. 1 part beads + 9 parts 0.2 um filtered water or PBS (100 microliter beads + 900 microliter diluent).
2. Load ~200 microliter diluted beads into a single cell or bulk cell cartridge.
3. Run analysis with the following Scatter Plot with Log/Log X/Y Scaling:

Instrument	Chart 1 (X/Y)	Chart 2	Chart 3	Chart 4	Chart 5	Chart 6
488/561	FSC1 x TSC	FITC/GFP x PE/ DsRed	mCherry x PerCP/PI	PE-Cy7 x PE-Cy5		
405/488	FSC1 x TSC	BFP x BV510	BV570 x BV605	FITC/GFP x PE/DsRed	PE-Texas Red x PerCP/PI	PE-Cy7 x PE/ DsRed

Expected Results



Single Cell Dispense Accuracy

Prepare a diluted aliquot of the Accuracy 10 micron beads (URFP001-100-10).

- 1 part beads + 9 parts 0.2 micron filtered water or PBS (100 uL beads + 900 microliter diluent).
- Load ~200 microliter diluted beads into a single cell cartridge.
- Run analysis with a Scatter, Density, or Histogram plot to identify the bead population.
- Insert a gate around the single population (chart or channel type does not matter).
- Dispense single beads at 1-2 events/sec onto a glass slide or into a 96 or 384 well plate and observe single bead deposition with a microscope (fluorescence is ideal).

Bulk Sort Purity Testing

Prepare a diluted aliquot of the Bulk Sorting Mix 6 micron Nile Red/10 micron Rainbow 80/20 mix (URFP-1006056-10).

- 1 part beads + 9 parts 0.2 micron filtered water or PBS (100 microliter beads + 900 microliter diluent).
- Load ~300 microliter diluted beads into a bulk sorting cartridge.
- Run analysis with a Scatter, Density, or Histogram plot with BFP or FITC/GFP to identify the two bead populations.
- Insert a gate around the bright 10 micron Rainbow bead population (i.e. this population is 20% of the Total)
- Collect ~1000-4000 target beads

Once bulk sorting is complete, insert the sorted sample into a single cell cartridge, and using the same gate from the bulk sort, and reanalyze the fraction sorted to determine sort purity. The purity of the sample can range from 70-90%

Chapter 7:

FAQ and Troubleshooting

Chapter Overview

- Frequently Asked Questions
- Troubleshooting
- Dark Camera Image- How to dry the Cartridge Holder

Frequently Asked Questions

Frequently Asked Question	Response
Does the instrument need calibration	Pressures are calibrated at the factory and when the instrument is installed. They are also re-calibrated during the preventative maintenance visits.
Does the laser need alignment?	The Pala is aligned at the factory and should not need alignment once installed.
What is the shelf life of the cartridge?	The cartridge is warranted for 1 year from time of purchase. The cartridges are hermetically sealed and gamma irradiated at the time of manufacturing.
Can cartridges be reused?	To ensure sterile conditions, it is best that new cartridges are used for each experiment. Additional cells of the same experiment can be loaded into the same cartridge but should be limited to one day of use.
Can the instrument be placed into tissue culture hood?	Yes, if sterile sorting is needed then the instrument should undergo the cleaning procedure and maintained with sterile reagents.
What is the minimum number of cells needed for analysis and sorting?	The minimum input of cells is 100 and the minimum recommended volume of cells loaded in the cartridge is 100 microliters.
What is the typical cell viability achieved after single cell sorting?	We typically see 40-70% viability but this is cell line and type dependent.
Can water be used as sheath buffer for single cell dispensing?	Yes, we actually recommend using sterile water because the cell is only in the single droplet of water for a very short period of time before it is deposited into a microtiter well filled with media.
Can water be used as sheath fluid for bulk sorting and enrichment?	We do not recommend using water for bulk sorting or rare cell enrichment. PBS or another suitable media/buffer should be used as sheath buffer during bulk sorting and enrichment.
What conditions will my cells be subject to in using the Pala?	Unlike conventional FACS that relies on high pressure to push the sheath and sample to form a consistent stream in the flow chamber, the Bio-Techne single cell dispensers utilizes microfluidics, on which a gentle positive pressure and negative pressure (20-30 times lower than that of FACS) are applied to guide the flow of cells. The entire microfluidics path where the sample is in contact is contained in the disposable cell cartridge and hence the amount of time the cells are exposed to the pressure is also much shorter than FACS.
What makes the Bio-Techne single cell dispensers not require the drop-delay calibration that's typically required for FACS	Bio-Techne's proprietary microfluidics design allows precise prediction of the delay between when the laser hits the cell and when the cell arrives at the high-speed valve opening. Therefore, drop-delay calibration by a user for each individual run is not required and the instruments are ready to sort upon completion of the automated initialization.

Frequently Asked Question	Response
What is the maintenance required for the Bio-Techne single cell dispensers?	The maintenance for the Bio-Techne single cell dispensers is light for the user. It is recommended for the user to flush the system with sterile ddH2O using the automated shutdown procedure after each use. If the instrument is going to be sitting for an extended period of time with no use, it is recommended to empty the sheath bottle and perform a shutdown to dry out the fluidics lines and empty the waste bottle prior to storage. Depending on what sheath buffers are used, our FAS may make recommendations to adjust the maintenance protocol. A software guided sterilization procedure that takes about 60 minutes is strongly recommended upon bringing the instrument into a sterile environment (e.g. tissue culture hood).
Can the Bio-Techne single cell dispensers be kept in a tissue culture hood for long term?	The Bio-Techne Pala single cell dispensers can be kept in a tissue culture hood for long term. Please avoid using UV light in the hood where the device is placed.
Can you set the instrument to dispense more than 1 cells per well? Is there a maximum?	The system Set Plate Map allows you to specify the number of cells to dispense in each well. Please be mindful about the volume capacity of your collection vessel because since 1 µL volume is added each time a single cell is dispensed using the single cell dispensing mode.
What is the size limit of cells that the Bio-Techne Pala single cell dispenser can sort?	The Bio-Techne single cell dispensers are designed to sort standard sized mammalian cells. The fluidic path can accommodate cells or particles of up to 45 microns in diameter. Please consult with your FAS if you have any concerns about the size of your cells/particles.
Do the Bio-Techne single cell dispensers require special sheath fluid?	No special sheath fluid is required. For single cell dispensing mode, water or PBS or culture medium of choice can be use. For for bulk sorting, PBS or culture medium of choice should be used.
How does the rare cell enrichment mode work?	The Bio-Techne proprietary rare cell enrichment mode allows the system to only register events that pass the fluorescent trigger threshold set by the user. This enables the system to run the sample at up to 150 million cells/mL input density 50,000 cells per second to quickly identify and enrich the rare population of interest.
What fold enrichment can I expect with the rare cell enrichment?	The rare cell enrichment mode is ideal for enriching rare populations of 0.1% or lower. Fold enrichment range can vary by the starting purity of the target population in the sample. Rarer target population is typically associated in higher fold enrichment. Internally we have seen 25-fold enrichment for samples with 0.01-0.1% target population. Actual fold enrichment may vary.
How many fluorescent colors can be detected simultaneously by the 2-laser system Pala?	There are 11 and fluorescent channels using 6 PMT detectors on the Pala 405/488 nm system. There are 9 on the fluorescent channels using 5 PMT detectors on the Pala 488/561 nm system.

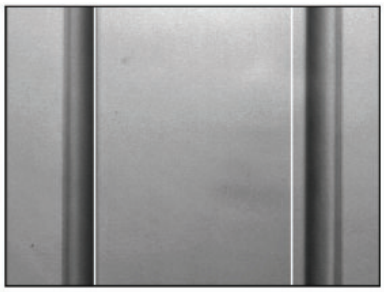
Troubleshooting

Issue/Observation	Troubleshooting
No Events Observed during "Analysis"	Ensure instrument was "Initialized" and waste line is dripping (>2 drops/sec) during initialization. Perform cartridge "Switch" and follow prompts. Check to ensure sample sufficient sample is in the cartridge and re-insert to repeat cartridge priming.
Waste Line not Dripping during Initialization (>2 drops/sec)	Ensure bottle caps and tubing connections are moderately tight. Ensure there are no obstructions or crimps in fluid lines (blue) or airlines (green – sheath, yellow – waste). Ensure cleaning cartridge is inserted correctly (notch facing into instrument).
Dripping Single Cell Cartridge Nozzle	See above "No Events Observed during Analysis" & "Waste Line not Dripping during Initialization". If cartridge holder is noticeably wet, gently dry with a sterile swab. Dispensing in Single Cell mode is not advised if cartridge nozzle is dripping.
Dark or Bright Camera Image	Perform the "Switch" cartridge function and then, using a sterile swab, gently dry out cartridge holder and reinsert cartridge.
Wet Cartridge Holder/Cartridge	Some liquid build up after multiple cartridges is normal and can be resolved with gently drying the cartridge holder with a sterile swab. .
No Liquid Dispensed during Single Cell Dispensing or Bulk Sorting	Ensure Gates are drawn on at least 1 chart and are colored blue. Repeat troubleshooting steps for "No Events Detected during Analysis" & "Waste Line not Dripping during Initialization". If the cartridge holder previously became wet due to a leak or insertion of a cartridge in the reverse orientation, use a swab to clear any liquid that may be trapped in the path from the cartridge nozzle to the plate/tube.
Large/Irregular Droplets during Single Cell Dispensing	Ensure the Single Cell Cartridge nozzle is not dripping prior to dispensing, see troubleshooting steps for "Single Cell Cartridge Nozzle Dripping" Ensure path from the cartridge nozzle to the plate/tube is clear of liquid.
Unexpected events populating with low fluorescence/FSC/TSC	Ensure that the sheath fluid is free from contaminants. Only use cell culture grade buffers that are sterile filtered through a 0.2 um media filter.
Fluorescent signal is low	Increase PMT gain
Error in saving data to specified file location	Ensure that there are no "spaces" at the end of the file name.
In Single Laser mode and setting a fluorescence "trigger", events are showing up below the "trigger" during "Analysis"	Turn "Analysis" off and back on again, repeat if necessary.

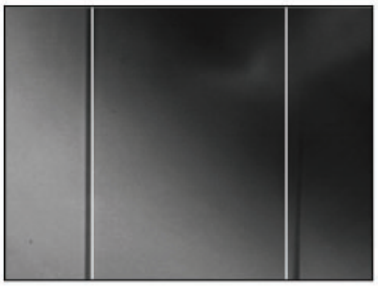
For any issues not resolved through these tips, please contact Technical Support at support@proteinsimple.com, visit https://bio-techne.com/support/instrument-support, or reach out to your Field Application Scientist.

Dark Camera Image- How to dry the Cartridge Holder

A silicon gasket in the cartridge holder seals the fluidic connections to the Single Cell or Bulk Cartridge to ensure proper flow of the sheath fluid through the cartridge nozzle and waste lines. When the cartridge is inserted and removed from the cartridge holder, the fluidic connections can release a small amount of sheath fluid that can block the optical port causing a dark camera image during alignment of the cartridge. This can happen after extended use when multiple cartridges are inserted and removed from the cartridge holder.



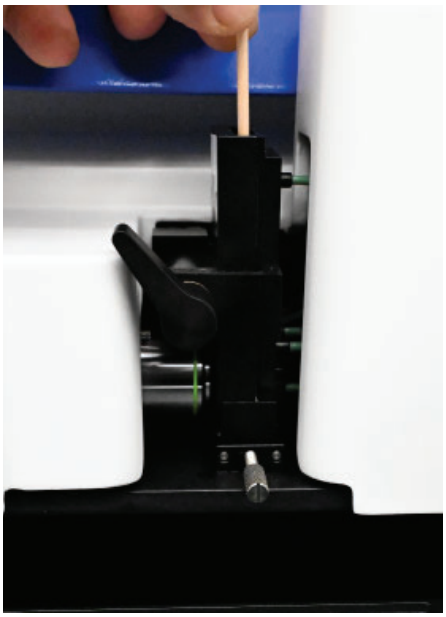
Normal- Dry Optical Port



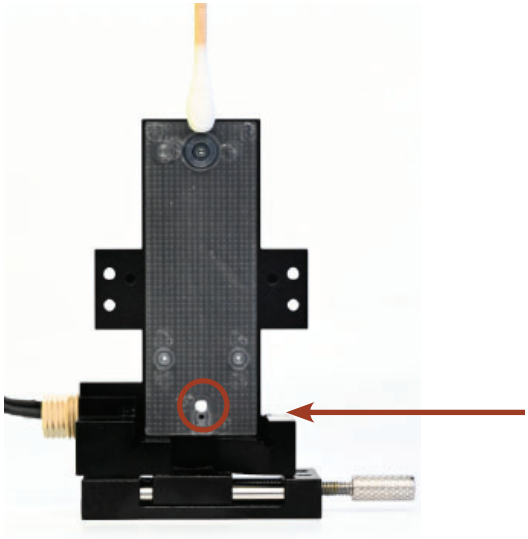
Fluid in the optical port

Fluid in the optical port can cause a loss of events during analysis. To remove the fluid and dry the optical port, a sterile swab needs to be inserted into the cartridge holder and brushed against the optical port to remove any residual fluid.

The optical port is located approximately 5 cm from the top of the cartridge holder.



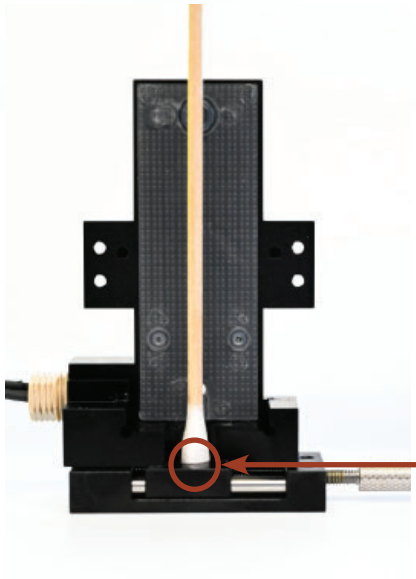
Inside view of the cartridge holder. The front portion of the cartridge holder has been removed to better illustrate the steps involved to dry the cartridge holder



Align the cotton swab to the center of the cartridge holder.

Optical Port is approximately 5 cm from the top of the cartridge holder.

Gently brush the swab around the optical port area to remove any excess liquid.



Push the swab through the bottom port to remove any additional liquid that may have accumulated in this area.

Chapter 8:

Appendix

Chapter Overview

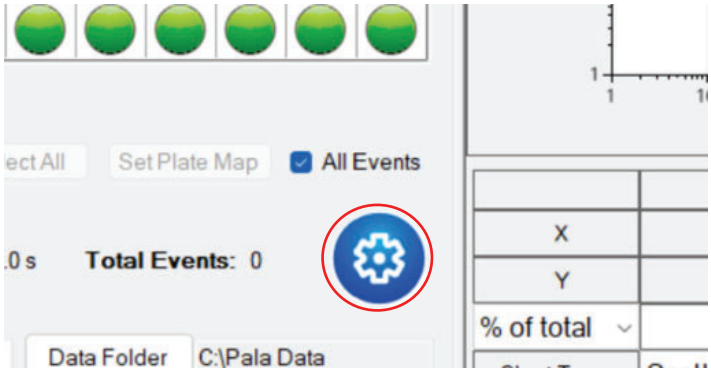
- Overview
- Accessing the Plate Control Menu
    - Plate Control Setup
    - Adjusting the A1 position
    - Laser and Valve Controls
- Sterilization Procedure
- Part Numbers for the Pala Instrument
- Contacting Bio-Techne

Overview

The appendix section will address additional instructions to optimize and set the correct settings for various microtiter format and types. In addition, this section will address the sterilization procedure in greater detail.

Accessing the Plate Control Menu

The Plate Control Menu menu be accessed by clicking on the Settings icon on the main screen and select the Plate Control Tab.



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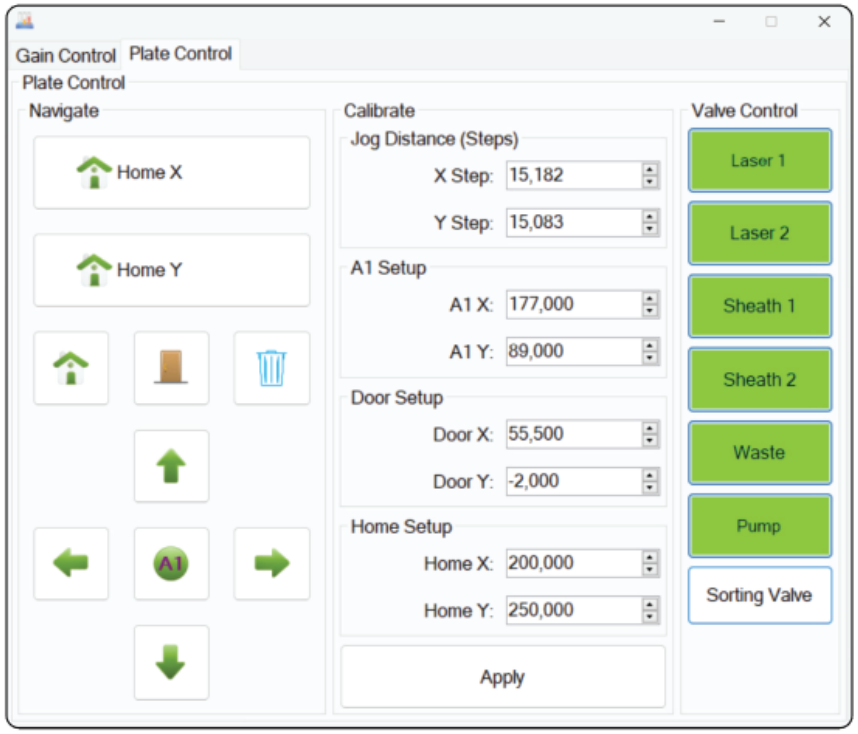


Plate Control Setup

Controls for the setting the correct spacing when using 96 well and 384 well microtiter plates. Please note that different manufactures may have different well to well spacing .

Switch or Value Box	Description	Notes	Default Value
Step Set Up	Defines the distance the plate stage will move in the X or Y coordinate in one movement. Adjusted in the factory for a standard 96 well plate.	Step_X=15182; Step_Y=15083	X=15182 Y=15083
A1 Set Up	Defines the X and Y coordinate of the A1 position. This is set in the factory to the coordinates of the A1 well of a standard 96 well plate.	Increasing the X value will move the A1 plate position to the Right while increasing the Y value will move the plate upward. Adjusting this value in increments of 500 is recommended.	X=176530 Y=86500
Door Set Up	Defines the X and Y coordinates of the "Door" position. This is adjusted such that the plate stage is 1.5 mm from the post		X=55500 Y=-2000
Home Set Up	Defines the X and Y coordinates of the "Home" positions.		X=200000 Y=200000

Adjusting the A1 position

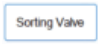
Insert a sample (preferably beads) into a single cell cartridge and go through the process to initiate analysis for the population of beads. .

1. Gate all data on the FSC x TSC chart. Click on the Settings Menu icon 

2. In the settings menu, click on the home position icon  to home the stage

3. Click on the Door icon.  to position the stage for loading of the plate

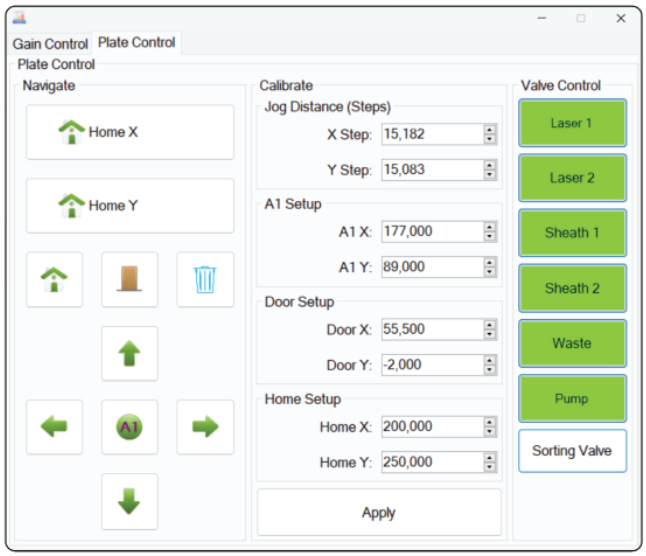
3. Place a 96 well plate with lid on the stage. Click the A1 icon  to position the plate to well A1.

4. Click Sorting Valve button ONCE  to dispense ONE bead, then click Left Arrow icon  .

5. Click Sorting Valve button ONCE  to dispense ONE bead, then click Left Arrow icon  .

6. Click Sorting Valve button ONCE  to dispense ONE bead, then click Left Arrow icon  ..

7. Click the Door icon  to position the plate back at the loading position. Check to see where the 3 droplets were dispensed and if they were in he center of the well. If the beads are in the middle of each of the 3 wells, then the A1 position is correct. .



A1 Setup

Increasing the X value will move the droplet to the **Left**

Decreasing the X value will move the droplet to the **Right**

Increasing the Y Value will move the **droplet Down**

Decreasing the Y value will move the droplet **Up**

If it is off in the X or Y direction, then change the A1 X and A1 Y numbers under A1 Setup. When making changes, repeat the previous step in dispensing 3 droplets until the 3 droplets are in the center of the 96 well plate.

DO NOT FORGET TO CLICK "APPLY"  after making changes to the Jog Distance, A1 Setup, Door Setup and or Hope Setup. Adjust in increments of ~500 to see a noticeable change in position. (1600 =1mm)

Increasing the X value will move the A1 plate position to the Right (thus moving the droplet leftward on the plate) while increasing the Y value will move the plate upward (i.e. toward the instrument) (thus moving the droplet downward on the plate).

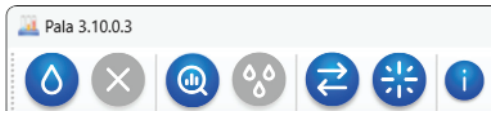
Laser and Valve Controls

Controls for the activation of the Lasers and Valves.

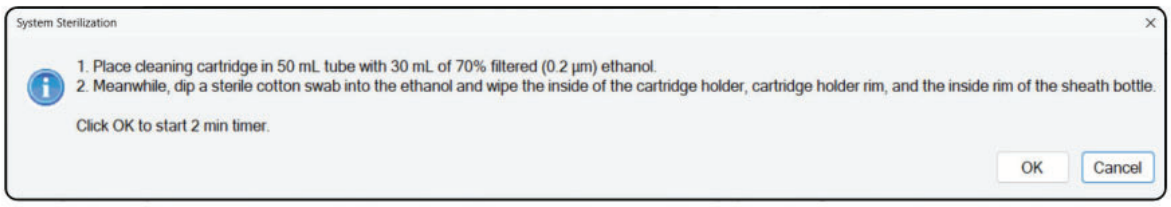
Button or Value Box	Description	Notes	Default Value
Laser 1	A manual toggle for turning laser 1 on and off. Laser 1 is the 488 laser on either the 405/488 or 488/561 systems.	Red = Off; Green = On.	n/a
Laser 2	A manual toggle for turning laser 2 on and off. Laser 2 is either the 405 or the 561 laser on the 405/488 or 488/561 systems respectively.	Red = Off; Green = On.	n/a
Sheath 1	A manual toggle for turning the sheath 1 valve open or closed.	Red = Closed; Green = Open.	n/a
Sheath 2	A manual toggle for turning the sheath 2 valve open or closed.	Red = Closed; Green = Open.	n/a
Waste	A manual toggle for turning the waste valve open or closed.	Red = Closed; Green = Open.	n/a
Pump	A manual toggle for turning the pump on or off.	Red = Off; Green = On.	n/a
HS Valve	The manual toggle for opening the high-speed valve for a period of time defined by the Spike and Hold settings.		n/a

Sterilization Procedure

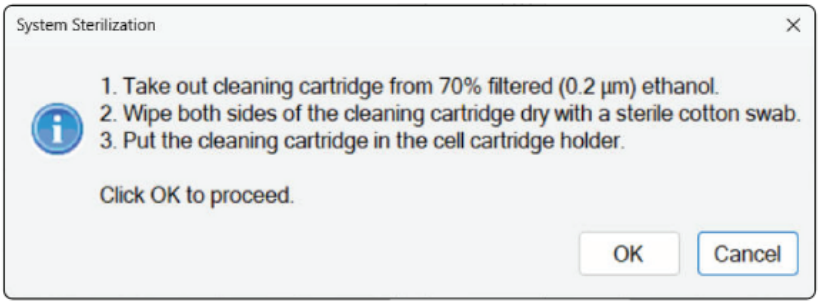
The procedure to sterilize the Pala system can accessed using the Sterilization Icon located on upper left corner of the main menu of the Pala software. After initiation of the sterilization procedure, the software will instruct the user through a series of prompts, the procedure to fully sterilize the Pala system. This full procedure will take about 60 minutes to complete



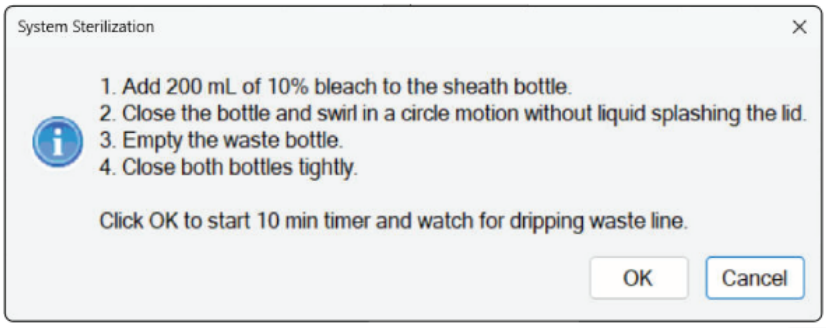
After clicking on the Sterilization Icon, a software prompt will appear with the following instructions below:



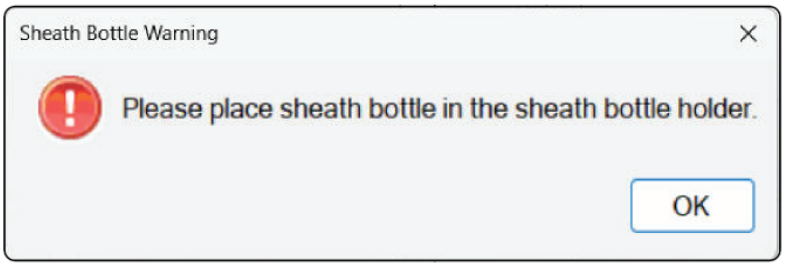
Step 2



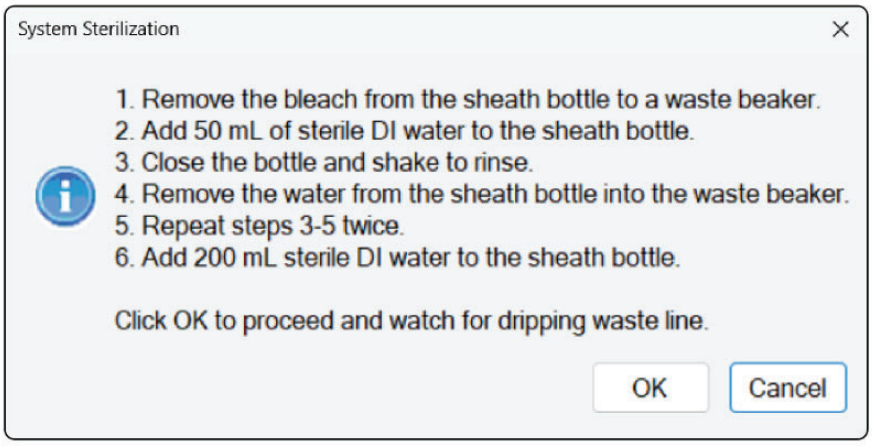
Step 3



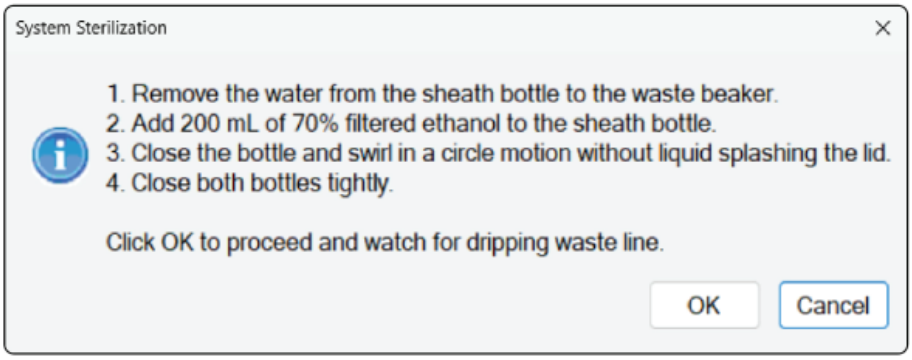
Step 4



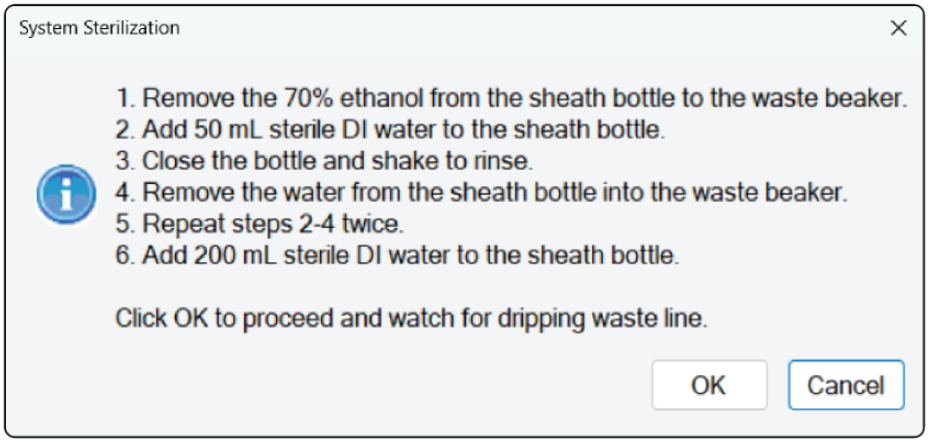
Step 5



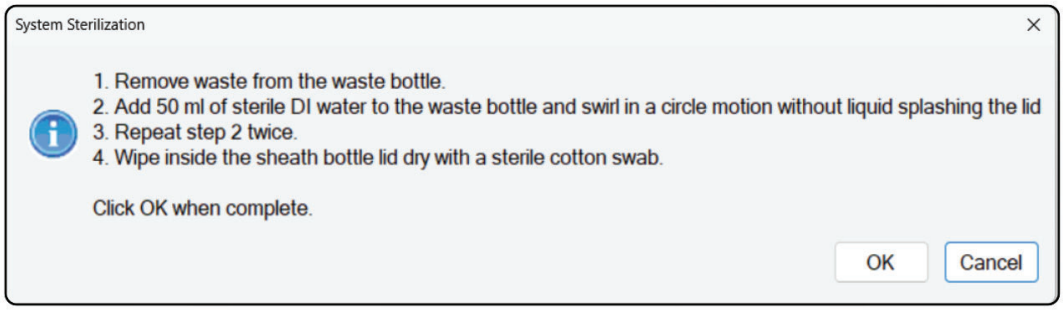
Step 6



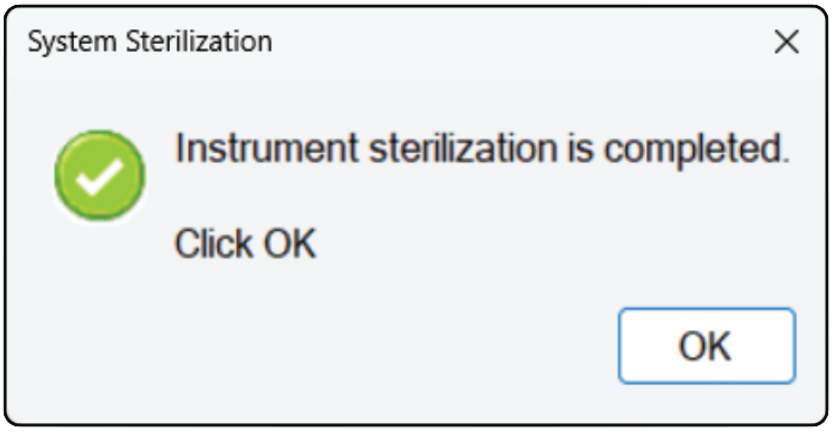
Step 7



Step 8


System Sterilization
1. Remove waste from the waste bottle.
2. Add 50 ml of sterile DI water to the waste bottle and swirl in a circle motion without liquid splashing the lid
3. Repeat step 2 twice.
4. Wipe inside the sheath bottle lid dry with a sterile cotton swab.
Click OK when complete.
OK
Cancel

Step 9


System Sterilization
Instrument sterilization is completed.
Click OK
OK

Part Numbers for the Pala Instrument

Part Number	Description
NI006	Pala 488/561 with workstation
NI007	Pala 405/561 with workstation
110-0021	Pala Workstation
NC003	Single Cell Cartridge (Box of 10)
NC101	Bulk Sorting Cartridge (Box of 10)
NC301	Cleaning Cartridge (Box of 10)
23015	Sheath Filter
23005	2-port cap
23020	Sheath/Waste 250mL Bottle
OP0084	Power supply (Pala)
OP0085	Power Cord
OP0086	USB 2.0 A to B Cable 6 ft
29011	Blue Flangeless Nut
29012	Yellow Flangeless Nut
29013	Green Flangeless Nut
405-1028	PCR Plate Adapter
CP0021	Bulk waste collector
A0033	2MM Adapter
27012	Cotton Tipped Wood Applicators (Sterile Q-Tips- Box)
OP0126	Bluetooth Mouse

Contacting Bio-Techne

The Pala instrument is supported by the ProteinSimple, a division of Bio-Techne.

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